# JOURNAL

of the

# RUBBER RESEARCH INSTITUTE

of

# MALAYA

VOLUME 15 SECOND PART 1957

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J. Rubb. Res. Inst.
Malaya 15 (1957)

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# *Stimulation of Bark Renewal of Hevea and its Effect on Yield of Latex*

P. DE JONGE

*Comparative effects, on bark renewal after tapping Heavea brasiliensis, of treatment with petroleum greases and palm oil, with and without 2,4-dichlorophenoxyacetic acid, were observed experimentally. Increases in latex yield were also observed and therefore studied intensively. Application of 2,4-D formulations above the tapping cut was compared with the standard practice of applying stimulant to scraped bark below the cut. Results of early experiments.*

Previous work (Beely & Baptist 1939) has shown that application of controlled quantities of palm oil to the freshly tapped hard bark of old rubber trees improved the subsequent renewal of the bark to a very significant degree. It was suggested that the more rapid and succulent development of the renewing bark following treatment of the tapped panel was due to some extent to the oily layer preventing excessive evaporation of water from the delicate inner cortical tissues exposed by the tapping process. The effect of palm oil was claimed to be due also to the presence of physiologically effective quantities of growth hormones which occur in most vegetable oils.

This pre-war work led to the setting up of an experiment in which were compared the effect on bark renewal of palm oil and several proprietary formulations when they were applied at monthly intervals to the thin film of bark left after tapping during the previous month. For this experiment buddings were used of clone AVROS 50, planted in November 1930 and tapped alternate daily on a half spiral cut.

Three different concentrations of 2,4-D were added to one of the proprietary formulations to test whether the growth stimulating effect of the substance could be transferred to the true cambium and produce an improved latex producing system in the bark. A strip of bark between ¾ to 1 inch wide was painted monthly using a flat paint brush half an inch in width. Care was taken that no overlapping of applications occurred as this has been shown to result in damage to the bark in the case of palm oil.

As the application of a yield stimulating mixture above the tapping cut was found to depress the yield (Chapman 1951), and as no yield recording has been made in the previously mentioned experiment (Beely & Baptist 1939) accurate yield records were taken throughout the three years duration of the experiment under review.

## DESCRIPTION OF EXPERIMENT

### *Treatments*

The following treatments were included in this experiment for monthly application above the tapping cut:

1 Control, no treatment to be applied
2 Standard Vacuum product 2295 C
3 Shell product Ensis 352
4 Shell product Otina C
5 Palm oil

COMMUNICATION 305

6 Palm oil plus 0.02% 2,4-D
7 Standard Vacuum product 2295 C plus 0.02% 2,4-D
8 Palm oil plus 0.1% 2,4-D
9 Standard Vacuum 2295 C plus 0.1% 2,4-D
10 Palm oil plus 1% 2,4-D
11 Standard Vacuum product 2295 C plus 1% 2,4-D
12 Stimulex

*Design of Experiment*

The experiment was assigned to the boundary rows of a density of planting experiment in which six different planting systems were compared, namely:

A 30 × 30 feet square = 48 trees per acre
B 20 × 20 feet square = 109 trees per acre
C 14 × 14 feet square = 222 trees per acre
D 12 × 12 feet square = 302 trees per acre
E 10 × 10 feet square = 435 trees per acre
F 20 feet triangular = 125 trees per acre

The plots of this density of planting experiment are arranged in a latin square design and the boundary rows of half of this area have been used for the experiment on stimulation of bark renewal, as in *Figure 1*. The figures in parentheses indicate the plot numbers of the density of planting experiment; the capital letters indicate the planting systems, as above; and the thick lines indicate the boundary rows used for the stimulation of bark renewal experiment. There are 18 rectangular shaped plots in the thick lined half of the density of planting experiment over which six different planting systems have been randomised. The boundary rows of each of these plots have been divided into four practically equal portions which form the plots of the stimulation of bark renewal experiment as shown in *Figure 2*.

| | | | | | |
|---|---|---|---|---|---|
| B | C | F | D (34) | A (35) | E (36) |
| A | D | C | B (28) | E (29) | F (30) |
| C | F | E | A (22) | B (23) | D (24) |
| D | E | A | C (16) | F (17) | B (18) |
| F | B | D | E (10) | C (11) | A (12) |
| E | A | B | F (4) | D (5) | C (6) |

*Figure 1. Layout of density of planting experiment.*

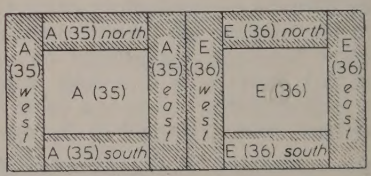


*Figure 2. Detail of two plots of Figure 1, showing the plots of the stimulation of bark renewal experiment, shaded.*

In this way 72 plots were obtained which allowed six replications of the 12 treatments. As there are six different densities of planting, each replication of a given treatment had to be allotted to a plot of different planting density. The design of this experiment allows a reliable comparison between bark treatments but there are no replications for the study of interaction between bark treatments and planting system. Furthermore the boundary rows do not truly represent the density of planting of the plots to which they belong because of interactions between the densities of the adjacent plots of the different planting systems. The number of experimental trees per plot is given in Table 1.

RECORDING

*Bark Renewal*

Bark measurements were taken at yearly intervals according to the following schedule (*Figure* 3). At the commencement of the experiment in December 1952 ($T_0$) one measurement was taken at the place where the tapping cut was estimated to have been in December 1951 (a). A second measurement was taken ½ inch above the centre of the actual tapping cut (b), thus indicating the thickness of bark left after tapping. Both positions a and b were clearly marked on the trees. After December 1952 ($T_0$) the treatments were applied at monthly intervals to the strip of bark left after tapping during the previous month, except for the control trees which received no treatment.

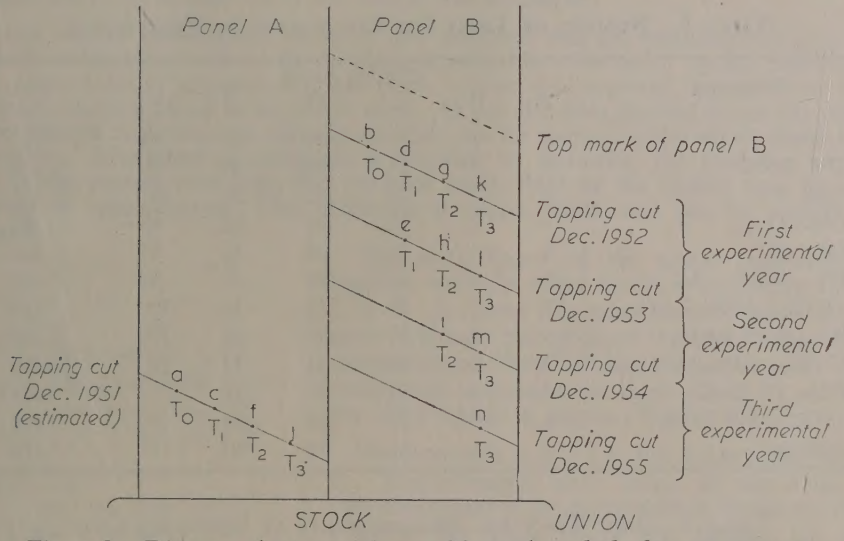


*Figure 3. Diagram of a tree giving positions of yearly bark measurements.*

The second set of bark measurements was taken in December 1953 ($T_1$) at one year after commencement of the experiment. Two of the measurements were taken as close as possible to positions a and b, but care was taken to keep clear of the wound reaction due to the earlier measurements. These measurements are labelled respectively c and d. A third measurement was taken at ½ inch above the position of the tapping cut at time $T_1$ (e) indicating the thickness of bark left after tapping in December 1953.

Application of treatments was continued throughout the second experimental year and a third set of bark measurements was taken in December 1954 ($T_2$), at positions f (close to c); g (close to d); h (close to e) and i (2 inches above the position of the tapping cut at the time ($T_2$). Measurement i therefore gives the thickness of bark of approximately two months renewal.

A fourth set of bark measurements was taken at the end of the third experimental year up to which time application of treatments had been continued without interruption. The time of measurements was December 1954 ($T_3$) and measurements were taken at positions j (close to f); k (close to g); l (close to h); m (close to i) and n (½ inch above the tapping cut at time $T_3$).

The anatomy of the renewal bark of six trees of each treatment was also studied under the microscope.

*Yield*

The yield was recorded by coagulation of the latex in the cup once a week. The cup lumps of each plot were then collected, dried for one month in an unheated drying shed and weighed. Recording was done for two months before the bark treatments were applied (pre-treatment recording) and was continued throughout the duration of the experiment. No corrections were made for the water content of the cup lumps after drying.

TABLE 1. NUMBER OF TREES PER REPLICATION OF TREATMENTS

| | Treatment | Planting system A | B | C | D | E | F | Total |
|---|---|---|---|---|---|---|---|---|
| 1 | Control | 6 | 7 | 28 | 35 | 22 | 8 | 109 |
| 2 | SVP 2295C | 6 | 9 | 23 | 33 | 37 | 8 | 116 |
| 3 | Ensis 352 | 6 | 7 | 25 | 32 | 31 | 10 | 111 |
| 4 | Otina C | 5 | 7 | 22 | 26 | 32 | 6 | 98 |
| 5 | Palm oil | 6 | 10 | 25 | 23 | 36 | 9 | 106 |
| 6 | PO + 0.02% 2,4-D | 7 | 8 | 26 | 29 | 30 | 8 | 108 |
| 7 | 2295C + 0.02% 2,4-D | 7 | 11 | 29 | 28 | 34 | 10 | 119 |
| 8 | PO + 0.1% 2,4-D | 6 | 8 | 27 | 29 | 30 | 5 | 105 |
| 9 | 2295C + 0.1% 2,4-D | 7 | 8 | 27 | 31 | 32 | 10 | 115 |
| 10 | PO + 1% 2,4-D | 6 | 8 | 20 | 30 | 31 | 9 | 104 |
| 11 | 2295C + 1% 2,4-D | 6 | 11 | 22 | 26 | 39 | 9 | 113 |
| 12 | Stimulex | 6 | 11 | 25 | 30 | 40 | 8 | 120 |

TABLE 2. BARK THICKNESS MEASUREMENTS AT ESTIMATED CUT LEVEL DECEMBER 1951

*Millimetres*

| | Treatment | a ($T_0$) Thick | Effect | c ($T_1$) Thick | Effect | f ($T_2$) Thick | Effect | j ($T_3$) Thick | Effect |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Control | 5.92 | 0 | 6.23 | 0 | 6.72 | 0 | 6.38 | 0 |
| 2 | SVP 2295C | 5.80 | –0.12 | 6.15 | –0.08 | 6.60 | –0.12 | 6.28 | –0.10 |
| 3 | Ensis 352 | 6.13 | +0.21 | 6.27 | +0.04 | 7.02 | +0.30 | 6.55 | +0.17 |
| 4 | Otina C | 6.97 | +0.05 | 6.07 | –0.16 | 6.60 | –0.12 | 6.53 | +0.15 |
| 5 | Palm oil | 6.15 | +0.23 | 6.37 | +0.14 | 6.90 | +0.18 | 6.38 | 0 |
| 6 | PO + 0.02% 2,4-D | 6.08 | +0.16 | 6.13 | –0.10 | 6.47 | –0.25 | 6.15 | –0.23 |
| 7 | 2295C + 0.02% 2,4-D | 5.83 | +0.09 | 6.40 | +0.17 | 6.80 | +0.08 | 6.37 | –0.01 |
| 8 | PO + 0.1% 2,4-D | 5.98 | +0.06 | 6.20 | –0.03 | 6.77 | +0.05 | 6.22 | –0.16 |
| 9 | 2295C + 0.1% 2,4-D | 6.02 | +0.10 | 6.18 | –0.05 | 6.80 | +0.08 | 6.38 | 0 |
| 10 | PO + 1% 2,4-D | 5.87 | –0.05 | 5.90 | –0.33 | 6.72 | 0 | 6.13 | –0.25 |
| 11 | 2295C + 1% 2,4-D | 6.02 | +0.10 | 6.17 | –0.06 | 7.02 | +0.30 | 6.38 | 0 |
| 12 | Stimulex | 6.13 | +0.21 | 6.18 | –0.05 | 7.02 | +0.30 | 6.33 | –0.05 |

*Girth*

Girth measurements were taken at a height of 60 inches above the union of all trees included in the experiment. Measurements were taken in December 1952 and 1955.

## RESULTS

*Bark Renewal*

The time and place of the various measurements are indicated in *Figure* 3. Measurements were taken with a Schlieper bark measuring gauge, which consists of a brass tube through which passes a steel plunger with a sharp bladed end. The blade, held horizontally, is pushed into the bark until resistance from the wood is met, when the depth of penetration can be read off to the nearest half millimetre.

Errors may be introduced if different field assistants take the measurements as much depends on the force with which the blade is pushed into the bark. In this experiment each year's series of measurement was taken by one field assistant, but different field assistants were employed in successive years. It was therefore decided to use the differences between measurements taken in any one year for comparing the effects of the bark treatments. The effect of treatments is obtained by deducting the thickness of the bark of the control trees from the thickness of the bark of the treated trees at each position of measurement. This difference is called 'effect' and can be positive or negative.

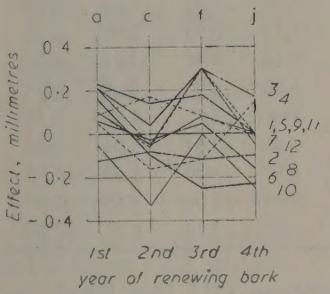


*Figure 4. Treatment effects at cut level estimated December 1951. The treatments, as numbered, appear on page 53.*

The mean figures of the actual measurements together with the effect figures are given in Tables 2 to 5. In these the measurements taken at the same level have been grouped together to allow for a comparison of effects between successive years. This comparison is brought out more clearly by plotting the effect figures in graphs (*Figures 4* to 7).

*Measurements* a, c, f, j — See Table 2 and *Figure 4*. Measurements were taken at the time of commencement of the experiment in December 1952 ($T_0$) at the place where the tapping cut was estimated to have been a year before, in December 1951. This place was determined by counting back twelve of the black dots such as are put on the trees on the first of each month on most estates with the object of keeping a check on bark consumption. The figures turned out to be rather high, suggesting that tapping had been shallow at the level of the cuts which at that time were situated low down on the panel on the opposite side. This should not interfere however, as measurements a, c, f and j, which were all taken at the same level, serve only to show whether the effect of bark treatments can be traced on untreated bark well away from the treated bark.

Any effect due to treatment should show up when bark thickness is plotted against time of measurement (years after first application of treatments). As, however, the actual measurements are not comparable the 'effect' figures are plotted along the ordinate. The effect of the control gives a horizontal zero line. Any treatment effect should then show up by a clear deviation from the control. Examination of the graph for measurements a, c, f and j (*Figure 4*) shows no regularity in any of the treatment lines, which are so closely grouped together that they cannot be clearly distinguished. They are all grouped closely along the control (0) line and therefore do not show any effect due to treatment of the bark at the opposite side of the trees.

*Measurements* b, d, g, k — Measurement b was taken ½ inch above the tapping cut at the time of commencement of the experiment ($T_0$) in December 1952 and measurements d, g and k were taken at the same level in December 1953 ($T_1$) December 1954 ($T_2$) and December 1955 ($T_3$). The mean actual bark thickness figures and the treatment effects are shown in Table 3 and the treatment effects are plotted in *Figure 5*. A first glance at this graph shows immediately that, whereas the treatment lines of *Figure 4* were scattered around the control line, all treatment lines are well above the horizontal control. The effects for treatments *10, 11* and *12* are outstanding. These treatments all contain a comparable concentration of 2,4-D (page 54), and have introduced a highly significant increase in bark thickness. All other treatments fall into one large group and show some increase in bark thickness. The trend of the lines suggests

TABLE 3. BARK THICKNESS MEASUREMENTS AT DECEMBER 1952 CUT LEVEL

*Millimetres*

| Treatment | b ($T_0$) | | d ($T_1$) | | g ($T_2$) | | k ($T_3$) | |
|---|---|---|---|---|---|---|---|---|
| | Thick | Effect | Thick | Effect | Thick | Effect | Thick | Effect |
| 1 Control | 3.45 | 0 | 4.63 | 0 | 4.78 | 0 | 4.52 | 0 |
| 2 SVP 2295C | 3.47 | +0.02 | 4.90 | +0.27 | 5.13 | +0.35 | 4.87 | +0.35 |
| 3 Ensis 352 | 3.40 | −0.05 | 5.02 | +0.39 | 5.48 | +0.70 | 4.95 | +0.43 |
| 4 Otina C | 3.57 | +0.12 | 5.28 | +0.65 | 5.30 | +0.52 | 5.02 | +0.50 |
| 5 Palm oil | 3.43 | −0.02 | 5.00 | +0.37 | 5.15 | +0.37 | 4.78 | +0.26 |
| 6 PO + 0.02% 2,4-D | 3.32 | −0.13 | 4.82 | +0.19 | 5.18 | +0.40 | 4.68 | +0.16 |
| 7 2295C + 0.02% 2,4-D | 3.33 | −0.12 | 5.17 | +0.54 | 5.50 | +0.72 | 5.25 | +0.73 |
| 8 PO + 0.1% 2,4-D | 3.60 | +0.15 | 5.27 | +0.64 | 5.60 | +0.82 | 5.07 | +0.55 |
| 9 2295C + 0.1% 2,4-D | 3.37 | −0.08 | 5.02 | +0.39 | 5.42 | +0.64 | 5.08 | +0.56 |
| 10 PO + 1% 2,4-D | 3.32 | −0.13 | 6.17 | +1.54 | 6.57 | +1.79 | 6.07 | +1.55 |
| 11 2295C + 1% 2,4-D | 3.57 | +0.12 | 7.22 | +2.59 | 7.40 | +2.62 | 6.60 | +2.08 |
| 12 Stimulex | 3.33 | −0.12 | 6.58 | +1.95 | 6.98 | +2.20 | 6.52 | +2.00 |

TABLE 4. BARK THICKNESS MEASUREMENTS AT DECEMBER 1953 CUT LEVEL

*Millimetres*

| Treatment | e ($T_1$) | | h ($T_2$) | | l ($T_3$) | |
|---|---|---|---|---|---|---|
| | Thick | Effect | Thick | Effect | Thick | Effect |
| 1 Control | 3.42 | 0 | 4.52 | 0 | 4.20 | 0 |
| 2 SVP 2295C | 3.35 | −0.07 | 4.75 | +0.23 | 4.42 | +0.22 |
| 3 Ensis 352 | 3.42 | 0 | 5.07 | +0.55 | 4.60 | +0.40 |
| 4 Otina C | 3.48 | +0.06 | 4.82 | +0.30 | 4.50 | +0.30 |
| 5 Palm oil | 3.67 | +0.25 | 4.87 | +0.35 | 4.60 | +0.40 |
| 6 PO + 0.02% 2,4-D | 3.65 | +0.23 | 4.82 | +0.30 | 4.50 | +0.30 |
| 7 2295C + 0.02% 2,4-D | 3.53 | +0.11 | 4.95 | +0.43 | 4.78 | +0.58 |
| 8 PO + 0.1% 2,4-D | 3.60 | +0.18 | 4.85 | +0.33 | 4.78 | +0.58 |
| 9 2295C + 0.1% 2,4-D | 3.38 | −0.04 | 4.85 | +0.33 | 4.82 | +0.62 |
| 10 PO + 1% 2,4-D | 3.63 | +0.21 | 6.35 | +1.83 | 6.15 | +1.95 |
| 11 2295C + 1% 2,4-D | 3.73 | +0.31 | 6.87 | +2.35 | 6.35 | +2.15 |
| 12 Stimulex | 3.77 | +0.35 | 6.32 | +1.80 | 6.25 | +2.05 |

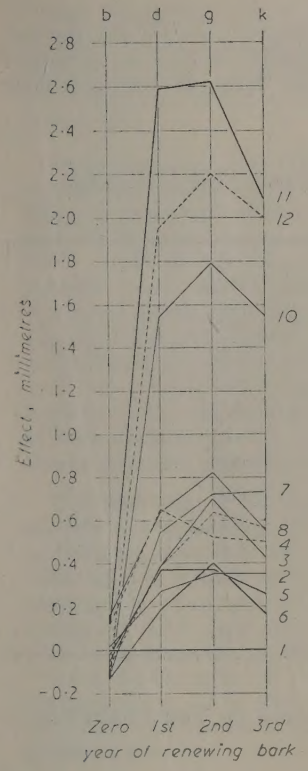


*Figure 5. Treatment effects at December 1952 cut level.*

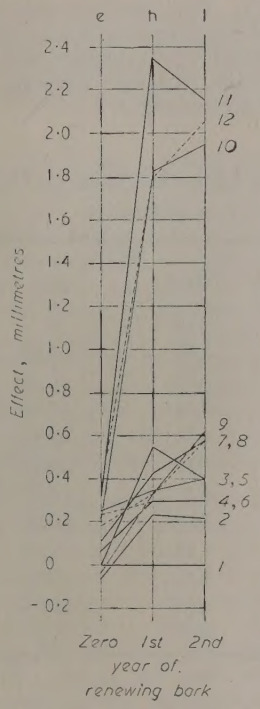


*Figure 6. Treatment effects at December 1953 cut level. See page 53.*

some additional effect from addition of 0.1% 2,4-D to palm oil or to Standard Vacuum product 2295 C.

It is doubtful, however, if the differences between these treatments would reach the level of significance as this trend is not invariably observed in all replications. The beneficial effect of an oil or grease treatment on the renewing bark is established beyond doubt and is most probably due to prevention of drying out of the delicate inner phloem tissue remaining after tapping. There is no suggestion that palm oil is better than the inert petroleum greases, which does not support the view that the physiological concentration of natural hormones in palm oil would account for the thicker renewed bark (BEELY & BAPTIST 1939).

None of the other treatments can compare with the outstanding effect of any treatment containing a 1% concentration of 2,4-D.

A further feature which can be clearly seen in the graph is the flattening out of the lines in the second year and the downward trend in third year. This observation is interesting as it suggests that almost all of the beneficial effect of bark treatment occurs in the first year of renewal and that in subsequent years the renewal of the control trees improves gradually relative to that of the treated trees, becoming actually better in the third experimental year.

The trend of the lines and the anatomical observations which will be discussed later suggest that no beneficial effect of treatments would be observed at the time when the renewing bark is tapped again.

*Measurements* e, h, l — Measurement e was taken one year after the commencement of the experiment at ½ inch above the tapping cut in December 1953 ($T_1$). Measurements h and l were taken at the same level in December 1954 ($T_2$) and December 1955 ($T_3$) respectively. The data are summarised in Table 4 and shown graphically in *Figure 6*.

Some effect due to treatment can already be observed at the time of measurement e ($T_1$) of the 14 days old renewing bark. Treatments *10, 11* and *12* (those containing 1% 2,4-D) start off from a slightly higher level than the others, thus suggesting an

almost immediate effect of 2,4-D. This was not noticed in the comparable series of measurements in *Figure 5*, where measurement b was also taken ½ inch above the tapping cut but just before the first set of treatments was applied so that no treatment effect could have shown up. This therefore supports the theory of the immediate effect of 2,4-D at the time of measurement e.

TABLE 5. BARK THICKNESS MEASUREMENTS AT DECEMBER 1954 CUT LEVEL

*Millimetres*

| Treatment | i ($T_2$) | | m ($T_3$) | |
|---|---|---|---|---|
| | Thick | Effect | Thick | Effect |
| 1 Control | 3.70 | 0 | 3.93 | 0 |
| 2 SVP 2295C | 3.67 | –0.03 | 3.80 | –0.13 |
| 3 Ensis 352 | 4.30 | +0.60 | 4.72 | +0.79 |
| 4 Otina C | 4.10 | +0.40 | 4.15 | +0.22 |
| 5 Palm oil | 4.22 | +0.52 | 4.08 | +0.15 |
| 6 PO + 0.02% 2,4-D | 4.12 | +0.42 | 4.07 | +0.14 |
| 7 2295C + 0.02% 2,4-D | 4.07 | +0.37 | 4.25 | +0.32 |
| 8 PO + 0.1% 2,4-D | 3.77 | +0.07 | 3.97 | +0.04 |
| 9 2295C + 0.1% 2,4-D | 4.13 | +0.43 | 4.37 | +0.44 |
| 10 PO + 1% 2,4-D | 4.55 | +0.85 | 5.40 | +1.47 |
| 11 2295C + 1% 2,4-D | 4.48 | +0.78 | 5.70 | +1.77 |
| 12 Stimulex | 4.97 | +1.27 | 5.80 | +1.87 |

TABLE 6. MEAN NUMBER OF LATEX VESSEL ROWS IN BARK OF THIRD RENEWAL AND VIRGIN BARK

*Samples taken at same level each year*

| Treatment | Age of bark | | | |
|---|---|---|---|---|
| | 1 yr. Dec. 1953 | 2 yr. Dec. 1954 | 3 yr. Dec. 1955 | Virgin Dec. 1955 |
| 1 Control | 8.1 | 10.0 | 10.8 | 22 |
| 2 SVP 2295C | 7.8 | 9.3 | 12.1 | 28 |
| 3 Ensis 352 | 9.8 | 10.1 | 11.7 | 23 |
| 4 Otina C | 9.1 | 11.0 | 12.3 | 29 |
| 5 Palm oil | 9.0 | 9.0 | 12.1 | 27 |
| 6 PO + 0.02% 2,4-D | 8.7 | 9.8 | 11.8 | 27 |
| 7 2295C + 0.02% 2,4-D | 10.1 | 9.1 | 11.8 | 27 |
| 8 PO + 0.1% 2,4-D | 9.3 | 9.5 | 12.0 | 24 |
| 9 2295C + 0.1% 2,4-D | 7.3 | 10.0 | 11.1 | 23 |
| 10 PO + 1% 2,4-D | 9.0 | 10.5 | 12.1 | 31 |
| 11 2295C + 1% 2,4-D | 9.7 | 10.7 | 13.4 | 27 |
| 12 Stimulex | 9.3 | 9.5 | 13.1 | 27 |

The treatment lines show the same pattern as observed in *Figure 5*, thus stressing the reliability of those observations. Almost all of the extra increase is obtained during the first year of renewal and several treatments show a downward trend in the second year.

*Measurements* i, m — These are shown in Table 5 and the treatment effect figures are plotted in *Figure 7*. Measurement i was taken in December 1954 ($T_2$) at 2 inches above the tapping cut and measurement m was taken at the same height in December 1955 ($T_3$). At position i renewing bark of approximately two months of age was measured, which served as a check on the suggested rapid effect of 2,4-D, found when measuring 14 days old renewing bark at position e (*Figure 6*). A marked effect due to treatment is observed on the two months old renewing bark for nearly all treatments, but especially for the formulations containing 1% 2,4-D. This observation therefore confirms that the effect of bark treatment is very rapid and that the observation made on the 14 days old renewing bark was not due to experimental error.

The high start of the Ensis 352 line is due to the fact that this mixture was replaced, as it ran out of stock, by a solution of 2,4,5-T in palm oil, reputed to be of 1% concentration. The outstanding effect of 1% 2,4,5-T formulations on yield and bark renewal as observed in other experiments makes the composition of this so called 1% 2,4,5-T in palm oil formulation rather doubtful. The downward trend of the three palm oil lines, respectively palm oil + 0.1% 2,4-D, palm oil + 0.02% 2,4-D, and palm oil, cannot be accounted for. The long storage of the formulations may have increased the free fatty acid content of the palm oil to an undesirable level.

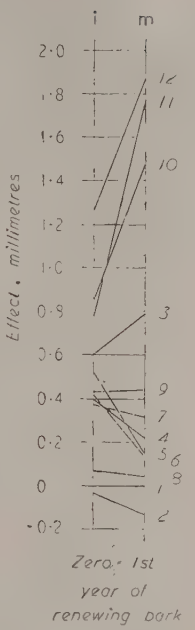


*Figure 7. Treatment effects at December 1954 cut level.*

To summarise the effect of bark treatments on the thickness of the renewing bark, all formulations have shown a beneficial effect on the thickness of the renewing bark; no additional effect has been observed as a result of the addition of 0.02% 2,4-D to Standard Vacuum product 2295 C or palm oil but some additional effect is suggested for the 0.1% concentration; all 1% concentrations of formulations containing 2,4-D (including Stimulex) have had a marked effect, which occurred mainly during the first year of renewal, in increasing the thickness of the renewing bark; the initial beneficial effect of bark treatments in increasing the thickness of the renewing bark occurs mainly during the first year of renewal and additional treatment seems to hinder subsequent renewal, so that trees not treated show a larger increase in thickness in subsequent years.

*Anatomy of the Renewed Bark*

It has been shown in the preceding section that several bark treatments have induced a significant increase in the thickness of renewing bark and the question arises whether this bark of increased thickness will have a better yield capacity than the non-treated thin bark of the control trees. A straightforward answer can be obtained by tapping the bark, but the thickness of the renewed bark of the control trees is as yet insufficient for tapping and this approach will have to be postponed for several years. An insight into the possible yield capacity can be obtained by microscopical examination of the anatomy of the renewing bark, assuming that within one clone the number of latex vessels is an indication of the yield capacity. This has been done at yearly intervals on six trees of each treatment and a summary of the count of latex vessel rows is given in Table 6.

Although there was a suggestion of a slight increase in the number of latex vessel rows in the treated bark, no significant difference could be established and it is concluded that none of the treatments had either a depressing or an improving effect on the cambium in the formation of latex vessels. The extra thickness of the treated bark occurs in the outer bark layers which contain no latex vessel and is very apparent in bark treated with formulations containing 1% 2,4-D (*Figure 8*).

These observations suggest that treatment with formulations for stimulating bark renewal will not result in an increased yield capacity of the renewing bark, but that the increased thickness may give a better support to the tapping knife. The occurrence of several phelloid like layers, however, make it probable that much of the extra thickness will flake off when the tapping knife cuts through the bark. This has already been experienced to a disturbing degree when bark samples for microscopical investigation were being collected.

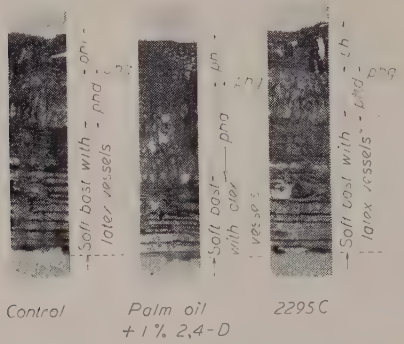
Soft bast with - phd - later vessels
Soft bast - phd with clear vessels
Soft bast with - phd - latex vessels
Control
Palm oil + 1% 2,4-D
2295 C

*Figure 8. Longitudinal section through three year old renewing bark.*
*phg phellogen phd phelloderm phl phellem*

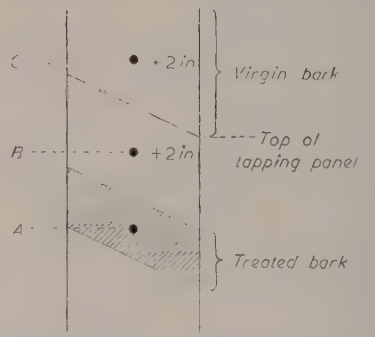


*Figure 9. Bark samples were taken at A at the centre of the treated bark, at B 2 inches above the treated bark, and at C 2 inches above the tapping panel.*

We have been fortunate in receiving a request, from the Director of Research of the Chemara Research Station at Seremban, for a microscopical examination of bark samples which had been treated with the yield stimulant Stimulex, which contains 2,4-D. We were given permission to publish the results of this experiment which links up closely with our stimulation of bark renewal experment under review. Buddings of clone PB 86 planted in 1938, had been treated in November 1935 with Stimulex on a three inch strip of renewing bark immediately above the tapping cut. In December 1955 the treated bark was stated to be approximately twice the thickness of the renewed bark immediately above the treated area, although showing no damage. Samples were collected from five treated and five control trees of similar girth at places indicated in the diagram in *Figure 9*. The results of our examination are summarised in Table 7. The detailed figures show a larger variation within than between treatments with regard to the number of latex vessel rings and the thickness of the soft bast and the suggestion of a slightly higher number of latex vessels and of a slightly thicker soft bast of treated trees is therefore not proven.

The 7.1 mm thick (9.3 to 2.2 mm) non latex containing layer of bark at the *A* position of the trees is expected to hinder subsequent renewal. This gradually lignifying periderm tissue is not expected to possess the ability to dilate which is shown by medullary rays in normally renewing bark. Deep cracks might therefore be expected to

appear if the activity of the cambium remains normal, but in our stimulation of bark renewal experiment it is already suggested that the increase in bark thickness of treated trees is slower in later years than that of the non-treated control trees. The samples taken at position *B*, two inches above the treated strip of bark, also show an increased thickness which shows that the growth stimulating effect of 2,4-D is not localised at the place of application.

A similar observation has already been made on trees treated with a yield stimulant on a three inch wide strip of scraped bark below the tapping cut, where it was found that the thickness of the renewing bark had been increased significantly. Microscopical examination of the bark samples did not reveal any change in the number of latex vessel rows (DE JONGE 1955).

It is interesting to note that the diameter of the latex vessels has not been changed by application of the yield stimulant so that the increased rate of latex flow cannot be explained by increase in diameter of the latex vessels. In time we may be in a better position to judge the merits of this method of stimulating bark renewal but at the present stage of research it would appear that no yield stimulants should be applied above the tapping cut on trees of which the renewing bark is to be tapped in future.

It is also doubtful whether treatment of a tapping panel with so called bark renewal stimulants which do not contain hormones or synthetic growth substances is an economical proposition except for wound treatment. Planting material at present recommended is capable of satisfactory bark renewal if proper upkeep and manuring of the fields are maintained, not only during the period of immaturity but also during later years of tapping. There would be need for a period of regular tapping panel treatment only in the case of outbreaks of fungal diseases. Such diseases can be controlled with the recommended fungicides if applied at a sufficient frequency to the newly tapped bark.

*Yield*

The yield was recorded by coagulation of the latex in the cups of all trees once a week. The cup lumps of each plot were collected monthly, air dried for one month and weighed. No correction has been made for the water content remaining after drying. Recording was done over two months before the experiment was commenced and was continued after application of the first set of treatments throughout the three years duration of the experiment.

TABLE 7. SAMPLES OF TWO YEAR OLD RENEWED BARK

*PB 86 Buddings, Treated and Control*

| Trees | Bark thickness, mm | | | Latex vessel rings | | |
|---|---|---|---|---|---|---|
| | *A* | *B* | *C* | *A* | *B* | *C* |
| Treated | 9.3 | 5.6 | 7.6 | 15.5 | 15.6 | 22.9 |
| Control | 4.1 | 4.3 | 7.9 | 14.7 | 14.4 | 23.0 |

| Trees | Soft bast, mm | | | Diameter of latex vessels at *A* |
|---|---|---|---|---|
| | *A* | *B* | *C* | |
| Treated | 2.2 | 2.2 | 3.9 | 19.6 to 21.6 mu |
| Control | 1.9 | 1.8 | 3.6 | 19.2 to 22.8 mu |

The soft bast contains the latex vessels. Sampling positions *A*, *B* and *C* are shown in Figure 9

TABLE 8. YIELD RECORDS

| Treatment | Pre-treatment mean yield gm/tree/tap | First year 1952-3 | | | Second year 1953-4 | | | Third year 1954-5 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Post-treatment mean yield gm/tree/tap | Adjusted post-treatment mean yield gm/tree/tap | Adjusted post-treatment as % of control | Post-treatment mean yield gm/tree/tap | Adjusted post-treatment mean yield gm/tree/tap | Adjusted post-treatment as % of control | Post-treatment mean yield gm/tree/tap | Adjusted post-treatment mean yield gm/tree/tap | Adjusted post-treatment as % of control |
| 1 Control | 20.6 | 15.6 | 15.0 | 100 | 18.0 | 17.3 | 100 | 23.4 | 22.7 | 100 |
| 2 SVP 2295C | 19.5 | 15.8 | 16.3* | 109 | 15.6 | 16.1 | 93 | 20.4 | 20.9 | 92 |
| 3 Ensis 352 | 18.4 | 13.4 | 14.9 | 100 | 14.9 | 16.4 | 95 | 22.4 | 24.1 | 106 |
| 4 Otina C | 22.9 | 18.7 | 15.9 | 106 | 19.7 | 16.8 | 97 | 26.4 | 23.4 | 103 |
| 5 Palm oil | 18.6 | 15.5 | 16.8** | 112 | 17.2 | 18.5 | 107 | 24.9 | 26.2 | 115 |
| 6 PO + 0.02% 2,4-D | 17.3 | 14.5 | 17.0** | 114 | 13.8 | 16.5 | 95 | 19.3 | 22.0 | 97 |
| 7 2295C + 0.02% 2,4-D | 20.7 | 17.0 | 16.3* | 109 | 18.6 | 17.9 | 103 | 24.1 | 23.4 | 103 |
| 8 PO + 0.1% 2,4-D | 20.1 | 18.3 | 18.2*** | 121 | 16.5 | 16.4 | 94 | 18.6 | 18.5 | 82 |
| 9 2295C + 0.1% 2,4-D | 19.0 | 16.3 | 17.3** | 115 | 18.2 | 19.2 | 111 | 22.4 | 23.4 | 103 |
| 10 PO + 1% 2,4-D | 16.9 | 22.5 | 25.5*** | 170 | 22.6 | 25.6*** | 148 | 24.9 | 28.1* | 123 |
| 11 2295C + 1% 2,4-D | 20.0 | 21.7 | 21.7*** | 145 | 21.5 | 21.5* | 124 | 26.8 | 26.8 | 118 |
| 12 Stimulex | 25.9 | 30.3 | 24.7*** | 165 | 28.7 | 22.9** | 132 | 35.1 | 29.1* | 128 |
| s.e. adjusted post treatment means | | | ±0.47 | | | ±1.18 | | | ±1.74 | |
| Min. 5% significant difference * | | | 1.3 | | | 3.3 | | | 4.9 | |
| Min. 1% significant difference ** | | | 1.8 | | | 4.4 | | | 6.6 | |
| Min. 0.1% significant difference *** | | | 2.3 | | | 5.8 | | | 8.5 | |

A summary of the yield records, statistically analysed, is given in Table 8. Highly significant increases in yield have been obtained during the first experimental year by those treatments which contained either 0.1 or 1% concentration of 2,4-D (including Stimulex). The best results were noted for the formulations containing 1% 2,4-D. Noticeable increases were also obtained with palm oil only and with palm oil plus 0.02% 2,4-D; although the addition of this low concentration of 2,4-D did not give an additional effect on yield. Both these treatments reached the 5% level of significance.

An increased yield at the 5% level of significance was obtained by treatment with Standard Vacuum product 2295 C and again the addition of the low 0.02% concentration of 2,4-D had no additional effect on yield. The yield stimulating effect of palm oil was not unexpected as it had been shown previously (BEELY & BAPTIST 1939) that increased yields were obtained if it was applied to the scraped bark below the tapping cut. This effect was attributed to the presence of hormones in this vegetable oil. Standard Vacuum product 2295 C however is a petroleum grease of the vaseline type of which an analysis has been carried out at the Department of Agriculture and Horticulture of the University of Bristol by Mr G. V. Coles, who reported:

> The product was a stiff brownish grease with an odour of refined residual petroleum. Over the temperature range 40°-50°C it gradually softened to a viscous brown oil with a green bloom. Analysis showed a trace of sulphur (about 0.01%) and ash (about 0.01%). The small amount of ash contained iron, copper, and zinc. Nitrogen, phosphorus, halogen, boron and mercury were absent. There was present only a trace (less than 0.01%) of acidic materials soluble in aqueous caustic soda. These were non phenolic in nature.

The product was analysed for C and H (at Oxford) and a commercial petrolatum ('vaseline') was included for comparison. The results were:

| | *Carbon %* | *Hydrogen %* | *Total* |
|---|---|---|---|
| 2295 C | 86.19 | 13.74 | 99.63 |
| Commercial petrolatum | 86.30 | 13.61 | 99.91 |

Dr L. C. Luckwill, also of Bristol, in order to find if any growth substances were included in this preparation which shows such a beneficial effect on wood healing, carried out the standard coleus leaf abscission test, which gave a negative result.

The reason for the observed increase in yield by monthly treatment of the bark above the tapping cut with Standard Vacuum product 2295 C would therefore appear to be the provision of a protection against drying out of the thin layer of bark remaining after tapping, which results in a more rapid formation of a cork cambium and subsequent periderm tissue. The trace of sulphur and ash are not expected to have influenced the yield. As however, the yield increase just reaches the minimum of the 5% significant difference level and the treatment concerned is one out of twelve, the yield increase is by no means established with certainty and as the possible effect is completely lost in subsequent years, it would serve no useful purpose to go further into this observation. The downward trend of response to treatment in successive years is striking and is well demonstrated in *Figure 11*, which shows the mean adjusted monthly yields of the three experimental years for treatments *1* and *12*, respectively control, and Stimulex.

This downward trend seems to be a feature of repeated applications of yield stimulants and has also been noted when they were applied at half yearly intervals on the three inch strip of scraped bark below the tapping cut on mature buddings and seedlings. With this method however, we have not observed a drop of yield to below the level of the non-treated control trees.

The application of formulations containing 1% 2,4-D (treatments *10*, *11* and *12*) in this experiment, in which application was made monthly above the tapping cut, has not resulted in a drop below control level either, and although there is distinct downward trend of the yield as time progresses, the yield is still some 20% above the control level during the third experimental year. It is not fully understood why treatment 9 (palm oil plus 0.1% 2,4-D) caused the yield to drop below that of the non-treated trees after giving a satisfactory increase during the first year.

The finding that it was possible to increase the yield of *Hevea brasiliensis* with monthly applications of yield stimulating formulations above the tapping cut has been followed up in further experiments on budded and seedling material and has been compared with the standard method of application of a yield stimulating mixture at intervals of six months to three inch wide strip of scraped bark below the tapping cut. As shown below, satisfactory yield increases have been obtained, but applications above the tapping cut invariably resulted in a very uneven bark renewal. An attempt was made to overcome this trouble by including a lower concentration of the growth substance in the carriers and later by using more suitable brushes for application.

The results of four experiments, one carried out on clonal seedlings and two on unselected seedling trees and one on budded trees follow.

*Clonal Seedling Experiment, Planted 1930* —The following treatments have been included:

- *A* Palm oil plus 1% 2,4,5-T monthly application above the tapping cut
- *B* Palm oil plus ½% 2,4,5-T monthly application above the tapping cut
- *C* Palm oil plus 0.1% 2,4,5-T monthly application above the tapping cut
- *D* R.R.I. yield stimulant monthly application above the tapping cut
- *E* Stimulex monthly application above the tapping cut
- *F* R.R.I. yield stimulant applied to 3 inch strip of scraped bark below the cut at 6 months intervals
- *G* Control

Yield records covering a period of six months are given in Table 9.

Table 9. Clonal Seedling Yield Records

*Mean monthly yields in grams per tree per tapping*

| Treatment | A | B | C | D | E | F | G |
|---|---|---|---|---|---|---|---|
| Pre-treatment (2 months) | 37.9 | 37.1 | 33.7 | 36.1 | 38.4 | 36.2 | 36.8 |
| 1st month | 51.1 | 46.2 | 34.9 | 49.6 | 57.8 | 67.8 | 40.4 |
| 2nd month | 54.5 | 53.6 | 42.9 | 58.9 | 55.2 | 53.8 | 44.4 |
| 3rd month | 49.3 | 51.1 | 41.6 | 57.5 | 57.8 | 44.7 | 42.0 |
| 4th month | 48.8 | 54.6 | 45.8 | 63.7 | 59.3 | 48.4 | 45.8 |
| 5th month | 45.2 | 53.4 | 46.9 | 58.9 | 61.4 | 49.6 | 49.2 |
| 6th month* | 38.3 | 46.8 | 42.7 | 52.6 | 50.2 | 41.5 | 39.5 |
| Mean 6 months | 47.9 | 51.0 | 42.5 | 56.9 | 57.0 | 51.0 | 43.6 |

* wintering

The superiority of the two commercial yield stimulating formulations, treatments *D* and *E*, is well brought out, and also the fact that application above the tapping cut has induced a higher overall yield than application below the tapping cut (treatment *F*) which latter gave a high initial increase followed by the familiar downward trend to slightly above control level. All treatments which were applied above the tapping cut, except treatment *C* which contained only 0.1% 2,4,5-T and failed to induce a yield increase, have resulted in a very irregular and undesirable bark renewal. This method of yield stimulation should therefore not be used if any interest is taken in renewing bark with regard to future tapping. It has the advantages that no bark scraping is required and that the yield trend is less irregular than with application of a yield stimulant below the cut.

No yield records are yet available from the experiment, which continues, using a new type of brush to enable a thinner application of the stimulant. The bark renewal appears now to be much better, but it is expected that there will be a decrease in yield response.

*Unselected Seedling Experiments, Planted 1930* — In these experiments monthly application of a yield stimulant above the cut is compared with half yearly application below the cut on trees tapped alternate daily either on a low cut in bark of second renewal or on a high cut in the virgin bark. The following treatments have been compared:

*H* S/2.d/2.100% tapping on low cut in bark of second renewal
*K* as *H*, with a stimulant below the cut once in 6 months
*L* as H, with monthly application of a stimulant above the cut
*M* V/2.d/2.100% tapping on a high cut in the virgin bark
*N* as M, with a stimulant below the cut once in 6 months
*O* as M, with monthly application of a stimulant above the cut

The yields during the first six months of this experiment are summarised in Table 10. This experiment again shows that yield stimulants applied below the cut on

Table 10. Comparison Between Stimulant Application Above and Below the Cut

*Mean monthly yields in grams per tree per tapping*

| Treatment | *H* | *K* | *L* | *M* | *N* | *O* |
|---|---|---|---|---|---|---|
| Pre-treatment (2 months) | 15.9 | 14.2 | 15.4 | 15.6 | 15.5 | 15.4 |
| 1st month | 18.0 | 36.4 | 16.9 | 18.6 | 49.6 | 27.3 |
| 2nd month | 22.0 | 26.8 | 21.9 | 20.6 | 34.7 | 27.1 |
| 3rd month | 22.2 | 19.0 | 27.0 | 21.8 | 31.0 | 27.7 |
| 4th month | 22.9 | 17.8 | 27.3 | 20.8 | 31.3 | 30.3 |
| 5th month | 22.4 | 18.9 | 28.0 | 22.3 | 28.1 | 32.1 |
| 6th month* | 21.1 | 18.2 | 26.9 | 20.2 | 25.2 | 29.0 |
| Mean 6 months | 21.4 | 22.9 | 24.7 | 20.7 | 33.3 | 28.9 |

* wintering

thin renewed bark lead to disappointing results, with the increase in yield lasting for a short time and the yield dropping below the level of trees not treated (treatment *H*).

Treatment above the cut on bark of third renewal also gave disappointing results, although the yield is consistently higher than that of the control trees. Treatment below the cut in virgin bark (treatment *N*) has given a distinct increase in yield and the response to treatment above the cut (treatment *O*) was also satisfactory. Under the conditions of this experiment it was three months before the yield level of treatment *O* caught up with that of treatment *N*. In other experiments this occurred in the first or second month after treatment. The bark renewal of the trees treated above the tapping cut was again very uneven.

*Further Unselected Seedling Experiment, Planted 1930* — The following treatments have been compared:

*P* S/2.d/2.100% tapping in bark of second renewal
*Q* V/2.d/2.100% tapping in virgin bark
*R* as Q, with a stimulant below the cut once in 6 months
*S* as Q, with monthly application of a stimulant above the cut

The results of the first six months of this experiment are summarised in Table 11. The superiority of the monthly treatment above the tapping cut is well brought out in this experiment and the yield on treatment *S* was similar to that of trees treated below the tapping cut as early as the first month of the experiment.

Table 11. Other Comparison Between Stimulant Application Above and Below the Cut

*Mean monthly yields in grams per tree per tapping*

| Treatment | *P* | *Q* | *R* | *S* |
|---|---|---|---|---|
| Pre-treatment (2 months) | 13.3 | 14.6 | 13.3 | 14.4 |
| 1st month | 13.0 | 17.6 | 31.7 | 32.1 |
| 2nd month | 17.6 | 17.9 | 20.6 | 26 5 |
| 3rd month | 12.1 | 14.7 | 18.1 | 27.1 |
| 4th month | 15.1 | 16.6 | 20.1 | 31.7 |
| 5th month | 15.0 | 15.8 | 18.5 | 31.3 |
| 6th month* | 14.7 | 15.1 | 17.1 | 32.3 |
| Mean 6 months | 14.6 | 16.3 | 21.0 | 30.2 |

* wintering

The treatments mentioned in Tables 10 and 11 all serve as control treatments in experiments carried out in these fields in which intensive tapping systems are tried out. These experiments will continue for several years and will therefore give a long term comparison between application above the tapping cut and below the tapping cut.

*Short Term Experiment on Mixed Buddings of AVROS 50 and Pil D65* — In the abovementioned experiments yield recording was carried out by coagulating the latex in the cup on each tapping day. The cup lumps were strung on a wire, hung on the trees and collected once a month for drying and weighing. In the experiment using budded trees of clones AVROS 50 and Pil D 65 recording was done by measuring the latex obtained at each tapping so that a day to day yield trend could be recorded. The typical yield trends for both treatments are demonstrated in the graph plotted from the data of this experiment (*Figure 10*), and are confirmed by the data of all the earlier experiments mentioned.

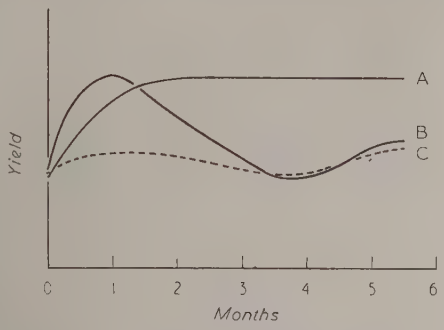


*Figure 10. Yield trend after application of Stimulex above or below the cut.*
A *above cut* B *below cut* C *control*

None of the experiments in which a comparison is made between applications above and below the cut has been running for more than six months. The effect of treatment above the tapping cut over a period of three years can be studied in the stimulation of bark renewal experiment under review. The commercial yield stimulating mixture Stimulex has been applied above the tapping cut, monthly without interruption throughout three years in the experiment, and the effect on the yield is given in *Figure 11*. The actual yield figures can be seen in Table 8, treatment 12.

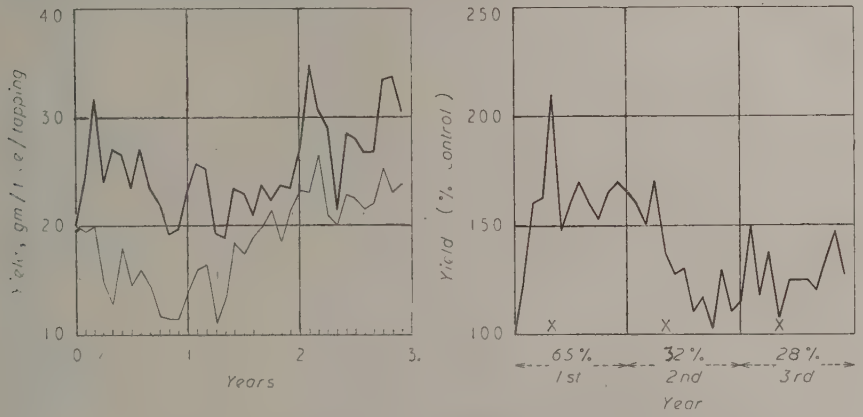


*Figure 11. Yield trend on application of Stimulex above the cut. Left: Lower plot is control. Right:* X *signifies wintering.*

The yield trend of both treated and control trees is irregular, but both follow approximately the same pattern. There is a downward response to the yield stimulant in the second year and a slow improvement appears in the third year. The downward response to yield stimulants has also been observed after repeated half yearly applications of stimulant to the scraped bark below the cut as illustrated in *Figure 12*. The histogram covers a period of three years during which six successive applications have been given at half yearly intervals. The lowered yield responses appear to be

characteristic of repeated yield stimulant applications, but there is evidence that further high yield increases will be obtained after change over of the tapping cut to the other side of the trees; similar decreases after successive applications of the stimulant may, however, be expected.

The yield has not dropped below control level in any experiment except the one which was carried out on low yielding old unselected seedling trees with very thin renewed bark (Table 10 treatment K).

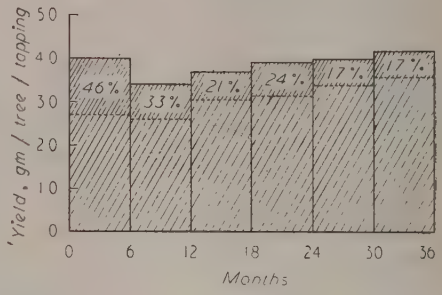


*Figure 12. Yield trend on application of Stimulex below the cut.*

From the above experiment it appears that monthly application of a yield stimulant above the tapping cut to the thin film of bark left after tapping during the preceding month is a highly satisfactory method of increasing the yield of rubber trees. There is much evidence to suggest that this method results in a higher overall yield increase than the standard method of half yearly application of the yield stimulant below the tapping cut. Long term comparisons between the two methods are in progress.

The thickness of the renewing bark becomes markedly increased if the stimulant is applied above the tapping cut, but the renewal is very irregular. We cannot yet, therefore, recommend this method of yield stimulation if further interest is to be taken in the bark left after tapping for future exploitation. Experiments are in progress to improve the method of application to overcome the disadvantage of irregular bark renewal.

*Girth*

From the above discussion of the effect of stimulants on yield, the question arises whether the increased yield level affects girthing. Girth measurements were taken at a height of 60 inches from the union of all trees included in the stimulation of bark renewal experiment in December 1952 and a second series of measurements was taken in December 1955. On these slow growing trees, the bulk of which occur in experimental planting systems where tree density is high, no influence of the treatments on the girthing of the trees could be detected.

Experiments are now in progress on vigorously growing young planting material. One of these experiments uses Prang Besar Further Proof seedlings planted in November and December 1949. Tapping commenced on 1 May 1956 and a yield stimulant was applied on 1 June to a three inch strip of scraped bark below the half spiral tapping cut. Girth measurements are being taken at six months intervals and the measurements taken in October 1956 showed the following girth increments from April to to October 1956: control trees 1.18 inches; stimulated trees 0.90 inches. The difference of 0.28 inches is significant at the 5% level.

Experiments with clonal and unselected seedlings have not revealed a retarding effect on growth due to repeated application of a yield stimulant either above or below the cut and it is recommended that the use of a yield stimulant be limited to material of which at least the virgin bark of the normal tapping panels has been tapped away. Treatment of bark above the tapping cut is not recommended if the treated bark is considered for tapping in future.

SUMMARY

An experiment has been described which was designed to compare the effects on bark renewal of vegetable and mineral oils, with and without the addition of 2,4-D, and the results are presented in tables and graphs. All treatments had an initial beneficial effect on bark renewal but highly significant results were obtained only from treatments containing 1% 2,4-D. It is apparent that the beneficial effects of the treatments occurred mainly in the first year and to a lesser extent in the second year. Subsequently the renewed bark of the control trees thickened at a greater rate.

The increased thickness of renewed bark as a result of the treatments appeared to be due solely to activation of the cork cambium. No increase of lactiferous tissue was noted.

Although the experiment under review was designed to compare the effects of the treatments on bark renewal, the increases in yield resulting from the treatments led to an intensive study of this aspect. The yield records from the experiment have been presented and discussed.

Notes have been presented on further experiments which extend the investigation into the possibility of employing yield stimulants above the tapping cut. These experiments are not yet complete but it seems unlikely that such applications can be usefully employed when the resulting renewed bark is to be tapped.

*I am indebted to Mr D. R. Westgarth for advice in the design of experiments and analysis of results. I express my thanks to Dr E. D. C. Baptist, former Head of the Botanical Division, who took an active part in planning and laying out the stimulation of bark renewal experiment. The able assistance of Mr T. S. Ayar who was in charge of the field work, of Mr Lee Choo Beng who compiled experimental data, and of Mr Chen Khyun Thai who compiled experimental data and prepared the bark sections for microscopical examination, is gratefully acknowledged.*

*Botanical Division*
*Rubber Research Institute of Malaya*
*Kuala Lumpur* *May*

REFERENCES

BEELY, F. & BAPTIST E. D. C. (1939) Palm oil diluent for tar oil fungicides and its effects on bark renewal of hevea. *J. Rubb. Res. Inst. Malaya* 9, 40.

CHAPMAN, G. W. (1951) Plant hormones and yield in *Hevea brasiliensis*. *J. Rubb. Res. Inst. Malaya* 13, 167.

DE JONGE, P. (1955) Stimulation of yield in *Hevea brasiliensis*. III. Further observations on the effects of yield stimulants. *J. Rubb. Res. Inst. Malaya* 14, 396.

# *Microflora of Hevea Latex*
# *1. Growth Media*

D. H. TAYSUM

*Methods have been devised for the culture of bacteria from fresh hevea latex, ammoniated field latex and ammoniated latex concentrate. The medium used is a modified Kligler's Iron Agar and permits the accurate evaluation of the numbers of bacteria present.*

PRELIMINARY STUDIES ON COMMON BACTERIAL CONTAMINANTS of hevea latex (EATON & GRANTHAM 1915, GORTER & SWART 1916) were followed by the isolation of twentyseven different strains of bacteria from fresh latex (DERNIER & VERNET 1917). Of these one is reported (CORBET 1929, 1930) to be common to all latices examined and is compared to *Bacillus pandora* Corbet, now considered a variant of *Bacillus polymyxa* Prazmowski, according to *Bergey's Manual of Determinative Bacteriology*. Slightly earlier (PRESCOTT & DOELGER 1927) a brief communication had also indicated the predominance of sporing organisms in latex and noted their activity towards carbohydrates. Studies on ammoniated latex (RHINES 1937) had served only to indicate the presence of anaerobes.

The above summation of bacteriologic studies on hevea latex prior to the Japanese invasion of south east Asia may give some impression of the vagueness of the position reached. While there was ample macroscopic evidence of bacterial activity in latex, ranging from natural coagulation and putrefaction to the production of bubbly sheet and spotted crepe, the organisms responsible were either unknown or so poorly defined as to be of little value in directing improvements in methods of latex preservation. This early work shows a pre-occupation with individual species which, in the light of recent experiments, seems difficult to justify; in general it is not possible to identify precisely many of the organisms originally described. There is no published evidence of any quantitative work having been done up to this time.

Renewed interest in latex bacteriology since the second world war culminated in an elegant series of reports by Mrs H. M. Overton in 1953 on the culture and isolation of bacteria from fresh hevea latex.* In addition ammonia resistance in bacteria was discussed (RHINES & MACGAVACK 1954) and interesting indications were given of bacterial effects on protein stabilised latex systems. The medium used in the former studies was frozen latex serum agar obtained by filter sterilising the thermolabile frozen latex serum and mixing with 6% aqueous sterile agar agar at 45°C. This medium is extremely difficult to prepare and equally tedious in use. However, by its inception, Mrs H. M. Overton (working in Malaya) and the author (working in England) were able to study the wide variety of fresh latex microorganisms. A detailed description of these will be given in a later paper; here it is only pertinent to note that the numbers present and the diversity of species clearly indicated that hevea latex was behaving as an excellent culture medium for bacteria, proliferation being as rapid as on the best laboratory media available.

A position was thus reached in which the numbers and species of fresh latex bacteria were known in some detail but in which sampling was distinctly limited by the diffi-

* Unpublished papers bound for reference at the Botany Library of the Imperial College of Science and Technology, London, and in the Research Archives of the Rubber Research Institute of Malaya, Kuala Lumpur.

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culty of medium preparation. In an industry which extends over a major portion of south east Asia and over a variety of ecologic conditions this is obviously a considerable deficiency. The reasons for attempting to use a medium closely resembling fresh latex are excellent and well founded but do not, of necessity, preclude the use of a synthetic medium. However, the failure of many simple laboratory media to grow more than a fraction of latex bacteria had already been noted and it was apparent that the design of a successful synthetic medium would follow a rather fuller consideration of the chemical constituents of fresh latex. It seemed that the failure of these synthetic media to provide cell for cell growth was not because of the paucity of latex as a culture medium but rather the reverse — because latex contains a variety of 'high energy' substrates as well as a diverse series of plant growth factors. The existence in hevea latex of relatively high concentrations of the polyhydric alcohols and their derivatives had been known for many years but more recently (ALTMAN 1940) attention had been drawn to the comparable high level of amino acid concentrations and to the presence of complex series of proteins (ROE & EWART 1942). There was further somewhat circumstantial evidence for the existence of lower molecular weight peptides and the high molecular weight nuclear proteins.

From the species present in latex and from experiments on the digestion of pure hydrocarbons and crepes it was known that the disperse hydrocarbon of latex was not a major substrate for latex bacteria and that a successful synthetic culture medium could be designed without its incorporation. It was thus apparent that a culture medium capable of satisfying the seemingly fastidious demands of the latex bacteria would simulate latex in its high concentration of amino acids, proteins, peptides and carbohydrates; somewhat tentatively it was expected that yeast extract would meet the growth factor requirements.

Three carbohydrates are present in fairly high concentration in hevea latex, quebrachitol (methyl-l-inositol), myo-(meso-)inositol and dextrose. Of these the last two are readily available commercially in sufficient purity for routine bacteriologic analysis. However, *Hevea brasiliensis* is one of the few plants which contain appreciable quantities of quebrachitol and even from this source it is difficult to obtain free from l-inositol; it is certainly not available in the quantities required of a routine test medium constituent. Quebrachitol has therefore to be replaced with some better known carbohydrate. From fermentation experiments on some of fifty strains of latex bacteria using fourteen carbohydrates, it was established that the fermentation of quebranchitol was unambiguously associated with lactose fermentation; i.e. that whenever quebrachitol exhibited acid production, lactose behaved similarly. The converse was not true; lactose fermentation is a much more widespread phenomenon than quebrachitol fermentation. This result was confirmed by sole source of energy tests, and, with some limitation of the number of species, by growth stimulation experiments. Quebrachitol was therefore replaced by lactose in the experimental test medium.

It is desirable that any medium to be employed in latex testing should be capable of distinguishing certain important metabolic processes. The first of these is acid production. Because of the instability of latex to low pH conditions, bacteria possessing the property of strong acid production are of dominant importance; the incorporation of a simple pH indicator in the growth medium permits the facile distinction of these. Gas production is of a similar technological importance since the presence of large numbers of organisms possessing this property invariably results in the production of bubbly sheet. It is thus obligatory to include such substrates (principally carbohydrates) as are required for the macroscopic manifestation of gas production. Finally it is necessary to detect the ability of organisms to produce hydrogen sulphide from constituents containing sulphur. Since the odour of latex is an important factor in its

marketing, a good latex or latex concentrate may be characterised by the absence of sulphide producing bacteria.

With the above considerations in mind it was not surprising that the medium finally designed bore a close resemblance to one already in routine use — Kligler's Iron Agar. It is thus best to consider the present medium as a modification of this for the specific purpose of growing latex microorganisms and not as an entirely new development.

## EXPERIMENTAL PROCEDURE

The percentage composition of the medium is given below — the closeness to Kligler's Iron Agar is apparent:

| | | RRI | KLIGLER |
|---|---|---|---|
| *1* | lab' lemco (or pure beef extract) | 0.3 | 0.3 |
| *2* | yeast extract | 0.3 | 0.3 |
| *3* | bacteriological peptone | 2.0 | 2.0 |
| *4* | sodium chloride | 0.5 | 0.5 |
| *5* | lactose | 1.0 | 1.0 |
| *6* | myo-(meso-)inositol | 0.3 | 0.0 |
| *7* | dextrose | 0.2 | 0.1 |
| *8* | ferric citrate | 0.03 | 0.03 |
| *9* | sodium thiosulphate (pentahydrate) | 0.03 | 0.03 |
| *10* | bromocresol purple | 0.005 | 0.005 |
| *11* | agar agar | 3.0 | 1.2 |
| | pH (distilled water solution medium) | 7.8±0.1 | 7.4 |

Agar agar is added to approximately 60% of the volume of water required for the final construction of the medium and is dissolved by steaming. Simultaneously items (*1*) to (*4*) are dissolved in approximately 15% of the volume of distilled water, warmed to 50 to 60°C. Items (*5*) to (*7*) are dissolved in a similar 15% volume and items (*8*) and (*9*) in the remaining 10%. These four solutions are mixed, the pH is adjusted to 7.8 (the samples of agar being cooled before testing) and bromocresol purple added as a solution in a few ml of absolute alcohol — this permits immediate dispersion in the medium. The mixture is then heated to 100°C and filtered hot through paper pulp to give a clear, blue purple, rigid, gell. The pH is checked before the final sterilisation. The medium is tubed in butted slope form for isolates and bulked in 120 ml quantities for routine latex testing.

The pH of 7.8 may need some explanation. For fresh latex, which directly after tapping approximates to neutrality, a lower pH is obviously desirable and media of pH 7.0 to 7.4 have been successfully employed. However for ammoniated latex and ammoniated latex concentrate, a medium of higher pH, 8.0 to 8.5, more closely simulates the latex environment. Higher pH values than these cannot be employed since mean generation times on such media become so high that visible colonies do not appear within a reasonable incubation period. Thus at least two media would be required to test these somewhat differing materials; this is an undesirable complexity in a test designed for use by relatively unskilled workers. Therefore the compromise pH of 7.8 has been adopted for testing these three materials. While the growth of fresh latex organisms is slightly slower at this pH than at 7.4, there is no detectable fall in the number of organisms declared viable.

Inocula from fresh latex for counting and isolation are obtained by serial dilution in isotonic saline (0.85% w/v). For early morning (say up to 11 a.m. — tapping time is 6.30 to 7 a.m.) a 1 ml inoculum from a 1 in 1,000,000 dilution of fresh hevea latex in saline will give deep plates exhibiting from 10 to 50 colonies per plate, which is ideal for isolation; for counting, a 1 ml inoculum from 1 in 100,000 dilution may give a

statistically better average at very early times after tapping. As the day progresses, the increase in numbers can be readily followed by further dilution to 1 in 10,000,000 (mid-afternoon) and 1 in 100,000,000 (early evening and after natural coagulation).

For ammoniated field latex (0.3%), 1 ml of a 1 in 100,000 dilution will generally suffice during the first two days (normal storage period). The mean generation time of organisms in this system is high enough to enable inocula from one chosen high dilution to be used for isolation.

It has been general practice for both fresh hevea latex and ammoniated field latex to set up a series of deep plates (approximately 20 ml of medium per plate) using 1 ml inocula of dilutions of the latex in saline ranging from $10^{-1}$ to $10^{-8}$. This procedure has enabled the isolation of all organisms, even those representing a vanishingly small proportion of the population. This is of importance as some of these small number strains survive factory processing remarkably well and reappear later as defects in the rubber; one example of this is *Serratia marcescens* Bizio, which never exceeds one thousandth of the total population but yet reappears as the inelegant spot disease of crepe. For counting purposes and for the isolation of dominant strains, it is usual to set up a series of plates at one chosen high dilution.

For ammoniated latex concentrate (approximately 0.7% w/w on the whole latex) the position is slightly different. The small number of viable bacteria present demands the use of higher inocula concentrations and the following series has been adopted as routine. Six sterile petri dishes are required for each latex concentrate to be tested. Into the first three are placed 5 ml, 2 ml, and 1 ml of the concentrate and rapidly 20 ml of the molten modified Kligler's Iron Agar (40°C) is added and the plates shaken vigorously until a uniform dispersion is obtained in each (the plates should be free from all latex 'flow lines'). Into the remainder are placed 1 ml each of a 1 in 10, 1 in 100, 1 in 1000 dilution of the concentrate in sterile isotonic saline; again 20 ml of molten medium is added per plate and dispersion achieved by vigorous shaking.

Incubation is at 30°C. Plates from fresh hevea latex can be read after a minimum of 18 hours, from ammoniated field latex after a minimum of 40 hours, and from latex concentrate after a minimum of four days. With badly prepared latex concentrate this lengthy period can be reduced to two to three days but as the latex concentrate approaches sterility the incubation time required for the growth of bacterial colonies becomes greater.

From time to time various additives to this medium have been tried differentially (e.g. latex serum extracts, proteins, quebrachitol). In no case has any significant increase in the number of viable organisms been detected. It may thus be supposed that the viable population of these latices is being uniformly sampled.

## EXPERIMENTAL RESULTS

The photographs show the efficacy of the medium in permitting the culture and isolation of latex bacteria. The plates in *Figures 1* and 2 are derived from fresh latex — the inoculum for each plate being 1 ml of the serial dilution indicated; the time of plating is approximately eight hours after tapping, mid-afternoon. Attention may be drawn to the dominance of strongly acid producing colonies and to the presence of strongly chromogenic bacteria (*Figure 1*, $10^{-3}$ and $10^{-4}$ plates); gas production is also of widespread occurrence but is not clearly shown in these photographs. *Figure* 3 is for ammoniated field latex (0.3% by weight); again the dominance of strong acid producing bacteria is apparent as is the complete failure of 0.3% ammoniation as a bactericidal process. *Figures 4*, 5 and 6 are for fully ammoniated latex concentrate (approximately 0.7% by weight) — the inocula are clearly indicated. In these cases it is necessary to

exercise some caution since the appearance of plates may differ widely according to the type of infection present. Chromogenic bacteria are particularly common but more important are the proteolytic *Bacilli* and the strongly acid producing anaerobes which are of variable occurrence. A rigid distinction should be made between chromogenic *Clostridia* and pigmented strains of *Serratia* and *Chromobacteria*. Thus these three last photographs should be considered merely as an indication of the growth of bacteria present in three particular cases and not as a general pattern to which all contaminated latices would conform. The problem of latex concentrate preservation will be dealt with in a later paper since the implications are of considerable practical importance to the latex industry; abbreviation of the results is not possible without considerable loss of accuracy.

The pH change required to give a detectable yellowing of the medium surrounding the colony is rather large (7.8 to 5.9) and thus detectable acid production on this medium corresponds to a considerable facility to produce strongly acidic conditions. This is desirable in fresh hevea latex and ammoniated field latex where the *Coliaerogenes* would otherwise complicate counting methods on acid producers. However, this drastic pH change may mean that in latex concentrate some of the organisms designated as not acid producing may produce sufficient acidic products to be detected by chemical methods if these organisms are present in large number. (Two such methods are in routine use, the first is the KOH number, the second is the volatile fatty acid evaluation). So far no example of this condition has been found but no doubt it can occur.

*This work forms part of a general research programme on the microbiology of hevea latex being carried out at the Rubber Research Institute of Malaya. The author wishes to thank the Staff of the Bacteriological Laboratory of the Imperial College of Science and Technology, London, for kindly interest and advice in the early stages of this work. The author is also indebted to Mr A. S. Dhillan for much practical assistance and friendly discussion.*

*A shorter description of this work has appeared in the Journal of Applied Bacteriology 19 (1956) 54.*

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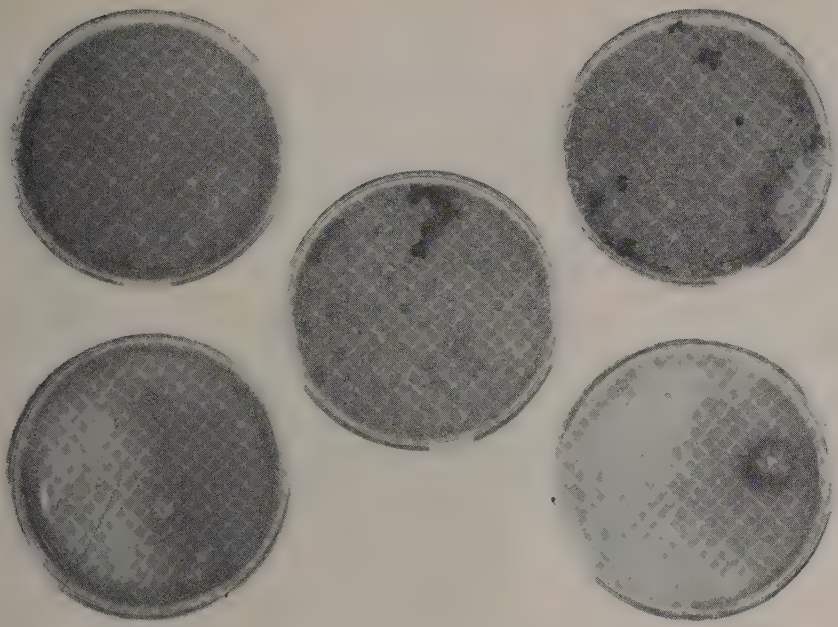
*Accepted December 1956*

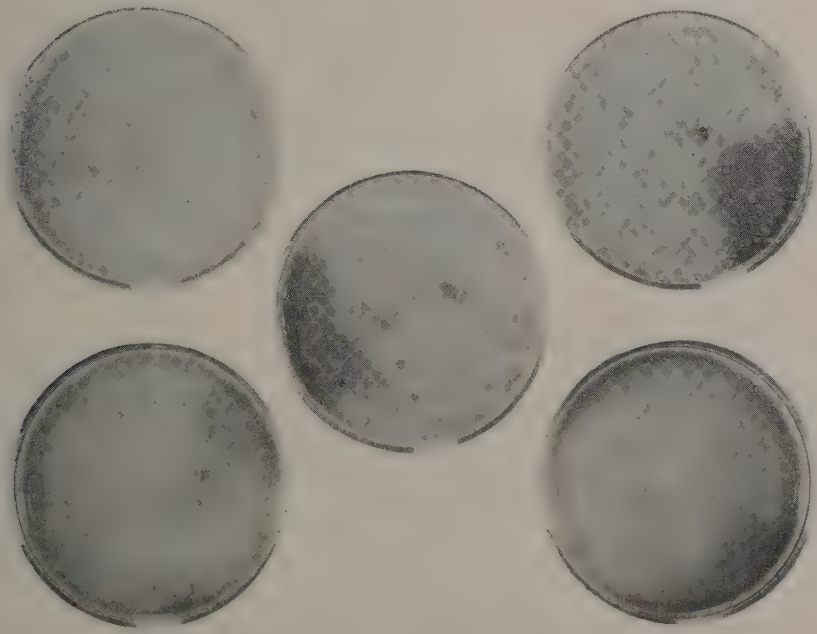
REFERENCES

Altman, R. F. A. (1940) *Arch. Rubbercult. 24*, 647.
Corbet, A. S. (1929) *The Natural Coagulation of Hevea Latex*, Kuala Lumpur.
Corbet, A. S. (1930) *J. Rubb. Res. Inst. Malaya 2*, 139.
Dernier, E. & Vernet, G. (1917) *C.R. Acad. Sci., Paris 165*, 123.
Eaton, B. J. & Grantham, J. (1915) *Agric. Bull. F.M.S. 4*, 26.
Gorter, K. & Swart, N. L. (1916) *Meded. Rubber-proefstation, W. Java 6*, 1.
Prescott, S. C. & Doelger, W. E. P. (1927) *J. Bact. 13*, 67.
Rhines, C. E. (1937) Private communication.
Rhines, C. E. & Maggavack, J. (1954) *Rubb. Age, N.Y. 75*, 852.
Roe, C. P. & Ewart, R. H. (1942) *J. Amer. chem. Soc. 64*, 2628.



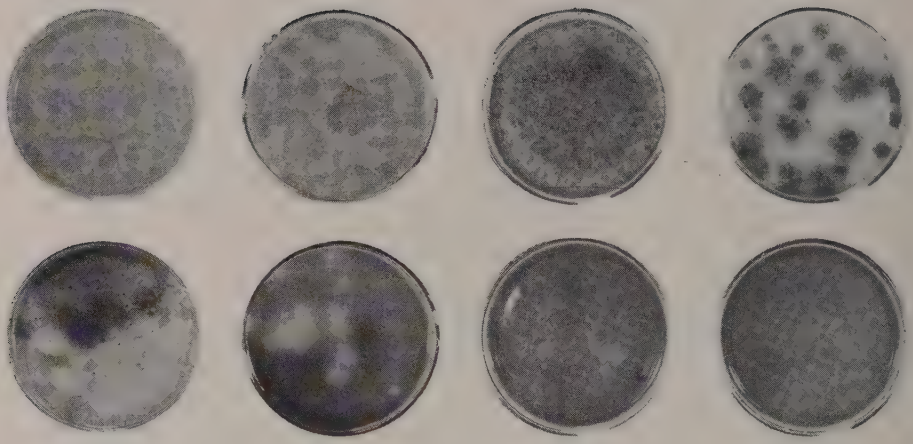
*1. Bacteria derived from fresh latex.*

$10^{-2}$ $10^{-3}$ $10^{-4}$ $10^{-5}$ $10^{-6}$



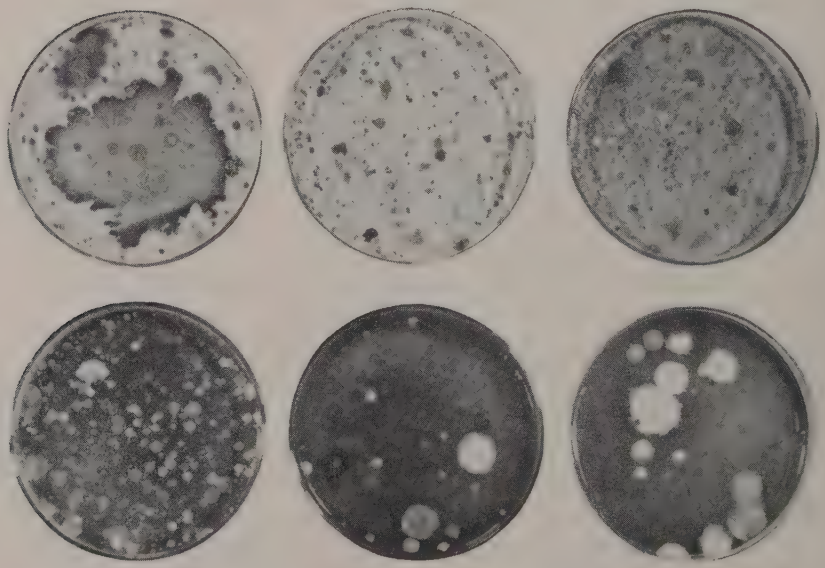
*2. Bacteria derived from another sample of fresh latex.*

$10^{-2}$ $10^{-3}$ $10^{-4}$ $10^{-5}$ $10^{-6}$



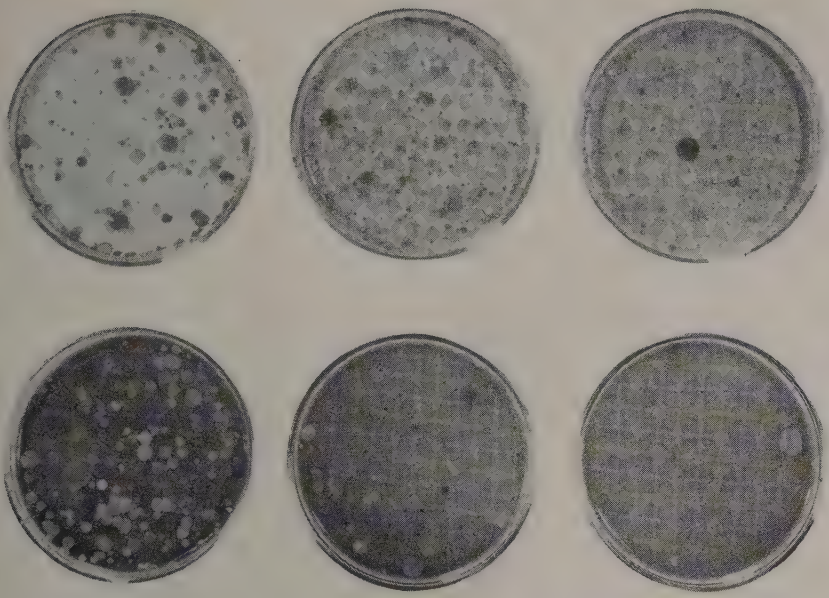
3. *Bacteria derived from two different samples (left and right) of ammoniated field latex.*

| | | | |
|---|---|---|---|
| $10^{-2}$ | $10^{-3}$ | $10^{-2}$ | $10^{-3}$ |
| $10^{-4}$ | $10^{-5}$ | $10^{-4}$ | $10^{-5}$ |



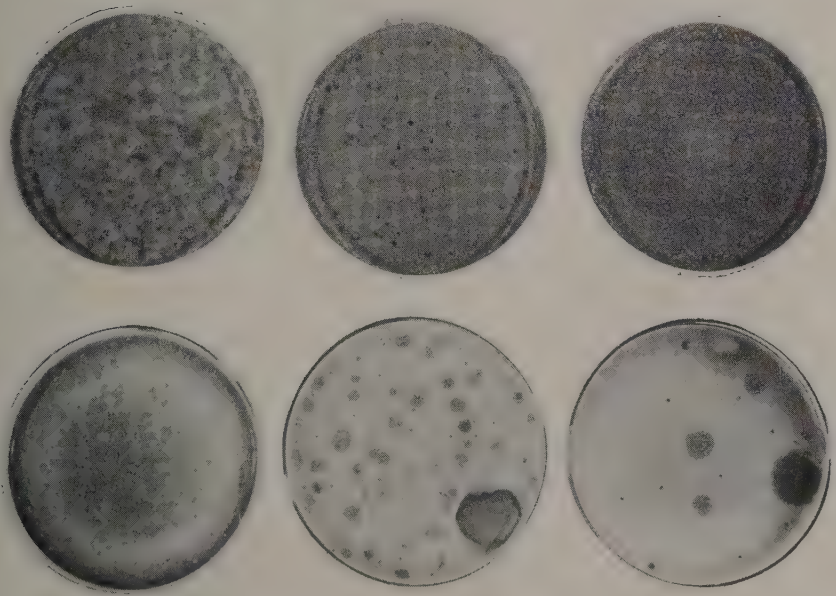
4. *Bacteria derived from ammoniated latex concentrate.*

| | | |
|---|---|---|
| 5 ml | 2 ml | 1 ml |
| $10^{-1}$ | $10^{-2}$ | $10^{-3}$ |



5. *Bacteria derived from another sample of ammoniated latex concentrate.*

| 5 ml | 2 ml | 1 ml |
|---|---|---|
| $10^{-1}$ | $10^{-2}$ | $10^{-3}$ |



6. *Bacillary outbreak in latex concentrate.*

| 5 ml | 2 ml | 1 ml |
|---|---|---|
| $10^{-1}$ | $10^{-2}$ | $10^{-3}$ |

# *Susceptibility of Hevea Seedlings to Helminthosporium Heveae Attack in Relation to their Nutrient Status*

E. W. BOLLE-JONES and R. N. HILTON

*Rubber seedlings were grown, exposed to infection by Helminthosporium heveae, in pots at three levels of nitrogen and two of calcium. Data for dry weight, number of leaflets, height, girth, and number of Helminthosporium lesions per leaflet are given for plants of each treatment. In addition, the results of leaf analysis for nitrogen, phosphorus, magnesium, potassium, calcium, and rubber are presented. It is concluded that the higher levels of nitrogen supply increased the number of lesions but the higher level of calcium did not. Chemical analyses suggested that the increased susceptibility at higher nitrogen levels might be associated with the relative reduction in the phosphorus and potassium concentrations within the laminae.*

THE RESULTS OF A STUDY OF BIRD'S EYE SPOT LEAF DISEASE, and its causal organism *Helminthosporium heveae*, were published in a preceding volume of this journal (HILTON 1952). It was established that this exceedingly widespread disease resulted from infection by the fungus of young leaves of plants up to two to three years old. Leaves that reached the stage of cuticle hardening were not infected, and plants over three years old were rarely seen with more than a few spots, despite the apparent susceptibility of their young leaves. Leaves of susceptible age on plants growing in the open were found to be almost invariably attacked throughout Malaya. As very young leaves were defoliated if infected heavily, plants could be sufficiently retarded in their growth to be too small for budding within the desired period of nine months. Intensity of attack was shown to depend on weather conditions and source of inoculum but the importance of soil was not fully assessed. All the observations and experiments were made on plants grown under field conditions, and it was noted that the effects of the disease were more severe on poorer, sandy soils. On more fertile soils the plants continued to increase in girth at a satisfactory rate despite heavy leaf spotting and even some defoliation. No attempt was made to distinguish between the separate effects of nitrogen, phosphorus, and potassium applications as this would have called for pot culture work as well as for a satisfactory method of measuring intensity of, as distinct from damage done by, the disease.

In 1954 an experiment was carried out (BOLLE-JONES 1955) in which the effects of different levels and sources of nitrogen on rubber seedlings growing in pot sand culture, in the open, were examined. The plants became infected by leaf fungi to varying degrees of intensity, and, although this interfered with the main purpose of the experiment, an excellent opportunity to study leaf disease fungi in relation to mineral nutrition of the host was presented. Superficial examination of the plants, which were in randomised positions over an area of about fifteen yards square, showed obvious differences in size and number of *Helminthosporium heveae* lesions between treatments. It was necessary to distinguish the effects of pot position and time of flushing

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of leaves in relation to infection, from the effect of nutrient treatment. To this end an observer, unaware of the treatment given, assessed the plants for severity as measured by an overall impression of lesion size and number. This showed that as the supply of nitrogen (either as ammonium or nitrate salts) increased so also did the severity of the infection; calcium appeared to have the reverse effect but this was not established with confidence. This first assessment, carried out in May was expressed by giving a score to each infected seedling without removing the leaves. A later assessment, carried out in August, related the score for each treatment to the number of examined leaves. All the leaves were removed from the penultimate storey of each plant and scored leaflet by leaflet. The total score was then divided by the number of petioles to give a value which would be independent of leaf number. The results of this assessment were examined statistically and it was found that no significant effects could be associated with variation of the levels of ammonium, nitrate or calcium ions. However, if the total score per plant was taken, a definite response to ammonium level was obtained, but as the number of leaflets also increased with ammonium level this effect could be attributed to the increase in the area of leaf surface exposed to infection and not to any increased susceptibility to attack.

Thus, difficulty was encountered in the assessment of the disease and it was decided to examine again the relationship between plant nutrient status and susceptibility to *Helminthosporium* by means of a separate experiment designed for this purpose. In this experiment the nitrogen and calcium supplies were varied and the assessment of the disease given as the number of lesions per leaflet. The experiment was not entirely successful and underlined the requirement for an assessment which would express severity of attack in terms of area destroyed per given area of lamina. The experiment did confirm, however, as anticipated on the basis of the 1954 experiment, that an increase in the incidence of the disease as measured by lesion number was associated with increased nitrogen level. This paper describes the observations and findings derived from this experiment.

## EXPERIMENTAL

Three levels of nitrogen were applied at each of two levels of calcium supply; this gave a total of six treatments, each of which was replicated six times. There were therefore 36 pots in the experiment; these freely drained pots were of five gallon capacity and contained sand which had been soaked for 24 hours in 5% (by volume of concentrated acid) hydrochloric acid and subsequently restored to neutrality before planting. On 21 June 1955 five seedlings of selfed clone Tjir 1 seedlings were transplanted into each pot and these were successively thinned out so that at the final sampling one plant only remained. Whole plants were removed for analysis in September 1955, and in March and August of 1956; the total dry weight per plant was recorded and the concentrations of rubber, nitrogen, phosphorus, magnesium, potassium and calcium in the laminae were determined.

The levels of nitrogen and calcium supplied were, in milligram equivalents per litre:

| | |
|---|---|
| $N_1$ : 0.51 m. eq. $NH_4^+$ + 0.99 m. eq. $NO_3^-$ | $Ca_1$ : 0.5 m. eq. $Ca^{++}$ |
| $N_2$ : 2.44 m. eq. $NH_4^+$ + 4.06 m. eq. $NO_3^-$ | $Ca_2$ : 4.0 m. eq. $Ca^{++}$ |
| $N_3$ : 4.31 m. eq. $NH_4^+$ + 7.19 m. eq. $NO_3^-$ | |

In addition all plants received the following concentrations of other nutrient ions (in miligram equivalents per litre) $PO_4^{---}$ 3.0, $Mg^{++}$ 2.5, $K^{+}$ 3.0, $Fe^{+++}$ 1.0, as well as a sufficiency of all the known micronutrient elements.

The experiment was laid out on a site exposed to full sunshine, some 100 yards away from the site of the original observations. It was necessary in September 1955, and in later months, to inoculate at least one plant per pot with spores of *Helminthosporium heveae* as the anticipated natural infection failed to develop.

## RESULTS

Clear symptoms of nitrogen deficiency (Bolle-Jones 1957) were obvious in the $N_1$ plants by March 1956. At this time differences in dry weight per plant, number of leaflets per plant and height, were beginning to appear (see Table, page 84). These differences became much more marked with time; in general the $N_3$ plants grew to be the biggest and most vigorous plants in the experiment.

An overall summary of the growth and analytical data is given in the Table. The analyses were carried out and the results expressed on the basis of the dried laminae, which excluded midribs and petioles. The trends and main effects were usually so consistent that it was not necessary to analyse statistically the raw data which fully supported the summary herein presented.

The dry weight, number of leaflets, height and girth of the $N_1$ plants were noticeably less than those of the corresponding $N_2$ plants. A smaller, but still distinct, disparity existed between the $N_2$ and $N_3$ plants especially in regard to girth and dry weight of plant.

As the nitrogen level increased so did the concentration of nitrogen found in the laminae but this was accompanied by a marked decrease in the concentrations of phosphorus, potassium and calcium. A decline in the concentration of rubber in the laminae, as the nitrogen level increased, was obtained in the March and October (not reported here) samplings. Nitrogen level did not consistently affect magnesium concentration but there was evidence that for the $Ca_1$ plants, increased nitrogen level increased the magnesium concentration but decreased it for the $Ca_2$ plants.

On 5 April mature leaves from the uppermost expanded storey were removed. The members of a pair of plants in each pot were labelled *A* or *B* at random; leaves removed from the *A* plants were examined by one observer and from the *B* plants by another. There were differences in growth between members of a pair, particularly in the high nitrogen pots. The results of this examination expressed as the total of the number of *Helminthosporium heveae* lesions per leaflet for each of the six treatments were as follows:

| Observer | $N_1$ $Ca_1$ | $N_1$ $Ca_2$ | $N_2$ $Ca_1$ | $N_2$ $Ca_2$ | $N_3$ $Ca_1$ | $N_3$ $Ca_2$ |
|---|---|---|---|---|---|---|
| *A* | 34.0 | 114.8 | 148.9 | 80.5 | 150.1 | 331.7 |
| *B* | 37.0 | 74.6 | 49.0 | 299.2 | 179.7 | 236.8 |
| Total | 71.0 | 189.4 | 197.9 | 379.7 | 329.8 | 568.5 |

Inspection of the totals suggested that an increase in nitrogen or calcium was associated with an increase in disease. Statistical treatment of this data gave the following results:

ANALYSIS OF VARIANCE

| Effects | Degrees of freedom | Mean squares | |
|---|---|---|---|
| N linear | 1 | 8477.43 | significant |
| N quadratic | 1 | 0.08 | not significant |
| Ca | 1 | 4033.52 | not significant |
| N × Ca | 2 | 150.90 | not significant |
| Error { pots within treatments | 30 | 1657.77 | |
| Error { plants within pots | 36 | 617.66 | |

TREATMENT MEANS

| Nutrient levels | $Ca_1$ | $Ca_2$ | Mean |
|---|---|---|---|
| $N_1$ | 5.9 | 15.8 | 10.8 |
| $N_2$ | 16.5 (±11.8) | 31.6 | 24.1 (±8.3) |
| $N_3$ | 27.5 | 47.4 | 37.4 |
| Mean | 16.6 (±6.8) | 31.6 | 24.1 |

Minimum 5% sig. diff. between 2 nitrogen means = 24.0
Minimum 5% sig. diff. between 2 calcium means = 19.6

TREATMENT EFFECTS

Linear effect of nitrogen = 26.6 ± 11.8 significant at 5% level
Quadratic effect of nitrogen = 0.2 ± 20.4 insignificant
Effect of calcium = 15.0 ± 9.6 insignificant

## DISCUSSION

The lesion counts clearly showed that increased nitrogen level increased the incidence of *Helminthosporium heveae* disease; calcium application did not yield any significant effect. As the lesion counts were expressed on the basis of leaflet number they were independent of the total number of leaflets borne per plant, which varied according to nutrient level (see Table). Unfortunately, however, the areas of the leaflets of the $N_2$ and $N_3$ plants were appreciably greater than those of the $N_1$ plants and it may be imputed that, in part, the higher lesion count of the $N_2$ and $N_3$ plants was due to a bigger leaflet area. There was not much disparity between the areas of the leaflets of the $N_2$ and $N_3$ plants but yet the increase in incidence of the disease was of the same arithmetic order between $N_1$ and $N_2$ as between $N_2$ and $N_3$. It may then be justifiably assumed that the increasing nitrogenous status of the plants was directly responsible for the increased incidence of the disease.

The chemical data (see Table) showed that, apart from increasing the nitogen concentration, increased nitrogen level decreased the concentrations of phosphorus, potassium and calcium. It seems unlikely that the effect on the concentration of calcium was directly related to the increased incidence of the disease as variation in the level of calcium supply produced no significant effect, as measured in terms of lesion count. However, it seems likely that the depression in phosphorus and potassium concentrations may well be linked with an increased incidence of the disease, possibly as a result of the accumulation of amino-acids (BOLLE-JONES 1955). To investigate

## Mean Values for Total Dry Weight, Number of Leaflets, Height and Girth of Plants, and Analyses of Laminae

*Laminae from top and second story leaflets were bulked for analyses. Analyses results expressed as percentages of dry lamina*

| | September 1955 | | March 1956 | | August 1956 | |
|---|---|---|---|---|---|---|
| | $Ca_1$ | $Ca_2$ | $Ca_1$ | $Ca_2$ | $Ca_1$ | $Ca_2$ |
| | | | TOTAL DRY WEIGHT PER PLANT (grams) | | | |
| $N_1$ | 6 | 6 | 49 | 49 | 83 | 88 |
| $N_2$ | 9 | 8 | 237 | 282 | 555 | 534 |
| $N_3$ | 10 | 8 | 257 | 311 | 621 | 652 |
| | | | NUMBER OF LEAFLETS PER PLANT (excluding top storey) | | | |
| $N_1$ | 12 | 12 | 17 | 18 | 16 | 16 |
| $N_2$ | 13 | 12 | 68 | 60 | 75 | 73 |
| $N_3$ | 12 | 13 | 67 | 86 | 88 | 90 |
| | | | HEIGHT PER PLANT (centimetres) | | | |
| $N_1$ | 47 | 45 | 106 | 116 | 145 | 148 |
| $N_2$ | 48 | 46 | 182 | 176 | 259 | 250 |
| $N_3$ | 47 | 46 | 185 | 187 | 281 | 285 |
| | | | GIRTH PER PLANT (measured in centimetres at a height of 7 cm above sand level) | | | |
| $N_1$ | — | — | 3.3 | 3.5 | 5.1 | 5.2 |
| $N_2$ | — | — | 6.0 | 6.2 | 9.8 | 9.7 |
| $N_3$ | — | — | 6.4 | 6.7 | 10.2 | 10.4 |
| | | | NITROGEN | | | |
| $N_1$ | 2.9 | 2.7 | 2.6 | 2.6 | 2.8 | 2.9 |
| $N_2$ | 3.4 | 3.6 | 3.1 | 3.0 | 2.8 | 2.9 |
| $N_3$ | 4.0 | 3.9 | 3.9 | 3.8 | 3.8 | 3.8 |
| | | | PHOSPHORUS | | | |
| $N_1$ | 0.34 | 0.36 | 0.51 | 0.66 | 0.88 | 0.76 |
| $N_2$ | 0.22 | 0.21 | 0.28 | 0.24 | 0.24 | 0.24 |
| $N_3$ | 0.26 | 0.28 | 0.26 | 0.24 | 0.24 | 0.27 |
| | | | MAGNESIUM | | | |
| $N_1$ | 0.18 | 0.17 | 0.25 | 0.28 | 0.24 | 0.23 |
| $N_2$ | 0.19 | 0.17 | 0.31 | 0.26 | 0.25 | 0.18 |
| $N_3$ | 0.22 | 0.16 | 0.29 | 0.28 | 0.25 | 0.20 |
| | | | POTASSIUM | | | |
| $N_1$ | 1.8 | 2.1 | 2.1 | 2.0 | 1.6 | 1.5 |
| $N_2$ | 1.4 | 1.4 | 1.5 | 1.4 | 1.2 | 1.2 |
| $N_3$ | 1.5 | 1.4 | 1.4 | 1.3 | 1.2 | 1.0 |
| | | | CALCIUM | | | |
| $N_1$ | 0.39 | 0.63 | 0.41 | 0.70 | 0.60 | 0.72 |
| $N_2$ | 0.33 | 0.47 | 0.25 | 0.55 | 0.29 | 0.56 |
| $N_3$ | 0.24 | 0.41 | 0.20 | 0.44 | 0.25 | 0.40 |
| | | | RUBBER | | | |
| $N_1$ | — | — | 0.62 | 0.55 | 0.42 | 0.47 |
| $N_2$ | — | — | 0.44 | 0.46 | 0.41 | 0.41 |
| $N_3$ | — | — | 0.46 | 0.43 | 0.46 | 0.40 |

the role of phosphorus and potassium in relation to susceptibility to *Helminthosporium heveae* disease future experiments must include varied levels of those nutrients.

The association of high nitrogen status with increased incidence of disease is well known for a number of crops (WINGARD 1941) but it is not suggested that control should be obtained by preventing vigorous growth. Under field conditions bird's eye spot disease causes proportionately more damage to poorly grown plants and our recommendations to estates are to maintain a well balanced manuring programme. The value of the observations presented here is that the susceptibility to attack under conditions of high nitrogen supply has been demonstrated under the precise conditions of sand culture and analyses of the plants have been recorded. They are intended to contribute to the discovery of the mechanism of disease resistance in the leaf of the rubber plant. Similar observations have been reported recently by workers on other crops. Nitrogen deficient barley leaves, which had hitherto resisted infection, became susceptible to mildew attack on being supplied with nitrogen (LAST 1953). A high N/K ration promoted greater susceptibility of rice plants to *Helminthosporium oryzae* infection (AKAI & MORI 1954). It seems likely that a similar explanation will be found to hold for this effect with such unrelated hosts and pathogens.

*We are indebted to Mr D. R. Westgarth for the statistical analysis.*

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*Accepted March*

REFERENCES

AKAI, S. & MORI, S. (1954) Studies on *Helminthosporium* blight of rice plants. II. Relation of the combination ratio of nitrogenous and potassium fertilizers to the susceptibility of rice plants to *Helminthosporium* blight. *Mem. Coll. Agric. Kyoto* 69, 1.

BOLLE-JONES, E. W. (1955) Comparative effects of ammonium and nitrate ions on the growth and composition of *Hevea brasiliensis*. *Physiol. Plant.* 8, 606.

BOLLE-JONES, E. W. (1957) Visual symptoms of mineral deficiencies of *Hevea brasiliensis*. *J. Rubb. Res. Inst. Malaya* 14, 493.

HILTON, R. N. (1952) Bird's eye spot leaf disease of the hevea rubber tree caused by *Helminthosporium heveae* Petch. *J. Rubb. Res Inst. Malaya* 14, 40.

LAST, F. T. (1953) Some effects of temperature and nitrogen supply on wheat powdery mildew. *Ann. App. Biol.* 40, 312.

WINGARD, S. A. (1941) The nature of disease resistance in plants. *Bot. Rev.* 7, 79.

# *Flame Photometric Determination of Potassium and Calcium and the Chemical Estimation of Phosphorus, Magnesium and Manganese in Leaves of Hevea*

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A BRIEF NOTE ON THE NATURE and accuracy of the methods of analyses used in this laboratory was given previously (BOLLE-JONES 1954). Since that publication requests have been received for a more detailed account of our methods of leaf analysis. The object of this paper is to provide a fuller account of some of these methods and to describe others which are now being used by us for routine leaf analysis. The observations reported below cannot be regarded as the results of original research yet they comprise an essential pre-requisite for the implementation of research and advisory programmes within the Soils Division. Our main aim has been to achieve an analysis, by three laboratory assistants, of about four dozen samples of leaf material for potassium, calcium, phosphorus, magnesium and manganese, in four days, instead of the usual six. To achieve this objective it was necessary to abandon the slower chemical methods for potassium and calcium determination in favour of the more rapid flame photometric procedures. This paper briefly describes and reports our experiences with the flame photometric methods of potassium and calcium determination, and the chemical methods of phosphorus, magnesium and manganese estimation, as used by us.

## PREPARATION AND DIGESTION OF HEVEA LEAF SAMPLES

The petioles as well as midribs are discarded, and the remainder of the laminae cut into small pieces and dried rapidly at 80°C in the presence of a powerful current of air. When perfectly crisp and dry, the samples are ground in a Wiley Mill until the leaf powder will pass through a 40 mesh sieve.

One gram of this powder is weighed into a 200 ml pyrex conical beaker and 10 to 15 ml of concentrated nitric acid (Analytical Reagent Grade, sp. gr. 1.42) added. The percentage moisture in the leaf powder is assayed by oven drying a separate sample. The conical beaker is covered with a watch glass and is gently heated on a steam bath until all the powder passes into solution, which generally appears straw coloured. Three millilitres of perchloric acid (Analytical Reagent Grade, sp. gr. 1.54) are added and the acid mixture heated on a hot plate; brown fumes are at first evolved to be followed later by copious white fumes which leave behind a colourless solution, measuring about 1 to 3 ml. After cooling, 25 ml of distilled water are added, the solution boiled and passed through a Whatman No 40 filter paper to be collected and made up to a 100 ml in a measuring flask. This solution, referred to hereafter as the 'wet digest', is analysed for potassium, calcium, phosphorus, magnesium and manganese. Under routine circumstances two dozen leaf samples can be wet digested every one and a quarter working days in this laboratory by one assistant.

### ESTIMATION OF POTASSIUM

Potassium is determined by a flame photometric procedure which is carried out directly on the wet digest, without difficulty. Such direct determinations have also been carried out by other workers (Toth, Prince, Wallace & Mikkelsen 1948). Potassium can be determined at a wavelength of 404 millimicrons (Blackwell, Yeager & Kraus 1953) or 767 millimicrons (Bauserman & Cerney 1953). The region of the latter wavelength is preferred as manganese, often present in large amounts in wet digests, interferes to a greater extent at 404 millimicrons (Roy 1956). All our determinations are carried out at 766.5 millimicrons. It is not found necessary to use an internal standard in order to reach the standard of accuracy given below. The Beckman D.U. spectrophotometer with flame attachment and oxyacetylene atomizer burner, but without photomultiplier, are used. The instrument controls are set as follows:

| | |
|---|---|
| Slit width .. .. .. | 0.05mm |
| Panel oxygen pressure .. | 10 p.s.i. |
| Panel acetylene pressure .. | 5 p.s.i. |
| Phototube knob .. .. | 'all in' (red phototube) |
| Load resistor switch at No 3 | (10,000 megohm) |
| Selector switch at .. .. | 0.1 |

The transmittance scale is set to 100% using the luminosity of a 300 p.p.m. potassium standard. The instrument setting is checked after every three determinations of 'unknown' digests by using a potassium standard whose strength is of the same order as that of the digests; it is reset, if necessary, using a 300 p.p.m. potassium standard. Usually the values obtained for the potassium concentration in leaves are within 10% of the values obtained by chemical means; with an experienced operator this deviation may be considerably reduced. Table 1 provides a list of flame photometric and chemical values, each pair estimated on the same wet digest, recently obtained from a series of determination carried out in a routine manner.

Very little difficulty is experienced in the flame photometric determination of potassium. Each single determination takes, under routine unhurried circumstances, not longer than two minutes (this average allows for the resetting of the instrument and periodic checking of standards during a 'run' of determinations). The chemical method of potassium estimation, formerly employed by us, involved a cobaltinitrite precipitation and subsequent premanganate titration and requires the full attention of one laboratory assistant for one and a half working days to complete about two dozen determinations, from the wet digest stage. The chemical method also requires a minimum aliquot, of the wet digest, of 25 ml whereas the flame photometric method requires no more than 7 ml.

### THE ESTIMATION OF CALCIUM

It proved much more difficult to evolve a routine flame photometric method of estimation for calcium than it did for potassium. Calcium estimation was rendered more difficult because of the well known interferences of phosphate, iron and aluminium ions (Hemingway 1956), apart from the evidence of our own trials which showed that cobalt enhanced, and chloride, nitrate and magnesium depressed, the luminosity of the calcium flame. The use of different wavelengths (422.7, 554 and 622 millimicrons) failed to overcome these interferences when the flame photometric estimation was carried out directly on the wet digest.

TABLE 1. COMPARISON OF POTASSIUM CONCENTRATION VALUES OBTAINED BY CHEMICAL AND FLAME PHOTOMETRIC METHODS OF ANALYSES FOR LEAF SAMPLES TAKEN FROM RUBBER PLANTS GROWN IN THE FIELD AND IN SAND

*The terms high and low are used only in relation to concentrations usually found in healthy laminae. All values expressed as percentage of oven dried laminae.*

| Description of Sample | Potassium % Chemical | Flame | K % Difference | % Error |
|---|---|---|---|---|
| **FIELD GROWN** | | | | |
| Normal | 0.54 | 0.49 | 0.05 | 9.3 |
| Normal | 0.62 | 0.63 | 0.01 | 1.6 |
| Normal | 0.72 | 0.79 | 0.07 | 9.7 |
| Normal | 0.80 | 0.79 | 0.01 | 1.2 |
| Normal | 0.96 | 1.02 | 0.06 | 6.2 |
| Normal | 1.22 | 1.27 | 0.05 | 4.1 |
| Normal | 1.81 | 1.79 | 0.02 | 1.1 |
| Normal | 1.80 | 1.86 | 0.06 | 3.3 |
| High in N (4.41%) | 1.31 | 1.39 | 0.08 | 6.1 |
| High in P (0.58%*) | 0.80 | 0.81 | 0.01 | 1.2 |
| High in Mg (0.51%) | 1.40 | 1.38 | 0.02 | 1.4 |
| Low in Mg (0.10%) | 0.50 | 0.52 | 0.02 | 4.0 |
| High in K (2.13%) | 2.13 | 2.15 | 0.02 | 0.9 |
| High in Ca (1.79%) | 0.49 | 0.47 | 0.02 | 4.1 |
| High in Fe (21339 p.p.m*) | 0.85 | 0.82 | 0.03 | 3.5 |
| High in Mn (309 p.p.m) | 1.18 | 1.27 | 0.09 | 7.6 |
| High in N (4.41%) and also in P (0.68%*) | 1.31 | 1.37 | 0.06 | 4.6 |
| High in N (4.19%) and also in K (2.19%) | 2.19 | 2.24 | 0.05 | 2.3 |
| High in P (0.56%*) and also in Ca (1.79%) | 0.49 | 0.48 | 0.01 | 2.0 |
| High in P (0.67%*) and also in Mg (0.51%) | 1.40 | 1.44 | 0.04 | 2.9 |
| Low in K (0.26%) and high in Mn (10820 p.p.m.*) | 0.26 | 0.24 | 0.02 | 7.7 |
| **GROWN IN SAND** | | | | |
| Normal | 1.22 | 1.24 | 0.02 | 1.6 |
| Normal | 1.08 | 1.10 | 0.02 | 1.8 |
| Normal | 1.08 | 1.12 | 0.04 | 3.7 |
| Normal | 1.13 | 1.16 | 0.03 | 2.6 |
| Normal | 1.07 | 1.12 | 0.05 | 4.7 |
| Normal | 1.12 | 1.15 | 0.03 | 2.7 |
| High in N (4.91%) | 1.36 | 1.48 | 0.12 | 8.8 |
| High in P (0.75%*) | 1.22 | 1.23 | 0.01 | 0.8 |
| High in P (0.73%*) | 1.08 | 1.12 | 0.04 | 3.7 |
| High in P (0.68%*) | 1.12 | 1.14 | 0.02 | 1.8 |
| Low in K (0.29%) | 0.29 | 0.28 | 0.01 | 3.7 |
| High in Mn (1165 p.p.m.) | 1.79 | 1.83 | 0.04 | 2.2. |
| High in Mn (16013 p.p.m.*) | 0.98 | 1.07 | 0.09 | 9.2 |
| High in P (0.64%*) and also in Mn (1165 p.p.m) | 1.79 | 1.83 | 0.04 | 2.2 |

* These high values are due to the deliberate addition of the respective elements to the wet digest.

TABLE 2. COMPARISON OF CALCIUM CONCENTRATION VALUES OBTAINED BY CHEMICAL AND FLAME PHOTOMETRIC METHODS OF ANALYSES FOR LEAF SAMPLES TAKEN FROM RUBBER PLANTS GROWN IN THE FIELD AND IN SAND

*The terms high and low are used only in relation to concentrations usually found in healthy laminae. All values expressed as percentage of oven dried laminae.*

| Description of Sample | Calcium % Chemical | Calcium % Flame | Ca % Difference | % Error |
|---|---|---|---|---|
| **FIELD GROWN** | | | | |
| Normal | 0.74 | 0.76 | 0.02 | 2.7 |
| Normal | 0.79 | 0.80 | 0.01 | 1.3 |
| Normal | 1.05 | 1.06 | 0.01 | 1.0 |
| Normal | 0.71 | 0.76 | 0.05 | 7.0 |
| Normal | 0.52 | 0.57 | 0.05 | 1.0 |
| Normal | 0.84 | 0.85 | 0.01 | 1.2 |
| Normal | 0.80 | 0.84 | 0.04 | 5.0 |
| Normal | 1.04 | 1.03 | 0.01 | 1.0 |
| High in Mg (0.44%) | 0.51 | 0.52 | 0.01 | 2.0 |
| Low in Mg (0.09%) | 1.14 | 1.14 | 0.00 | 0.0 |
| **GROWN IN SAND** | | | | |
| Normal | 0.62 | 0.66 | 0.04 | 6.5 |
| Normal | 0.73 | 0.74 | 0.01 | 1.4 |
| Normal | 0.65 | 0.64 | 0.01 | 1.5 |
| Normal | 0.72 | 0.73 | 0.01 | 1.4 |
| Normal | 0.56 | 0.57 | 0.01 | 1.8 |
| Normal | 0.59 | 0.64 | 0.05 | 8.5 |
| Normal | 0.65 | 0.65 | 0.00 | 0.0 |
| Normal | 1.11 | 1.10 | 0.01 | 0.9 |
| Normal | 1.08 | 1.09 | 0.01 | 0.9 |
| Normal | 0.40 | 0.40 | 0.00 | 0.0 |
| Normal | 0.91 | 0.85 | 0.06 | 6.6 |
| High in N (5.39%) | 0.68 | 0.70 | 0.02 | 2.9 |
| High in P (0.45%) | 0.37 | 0.40 | 0.03 | 8.1 |
| High in P (0.51%) | 0.54 | 0.56 | 0.02 | 3.7 |
| High in P (0.49%) | 0.71 | 0.68 | 0.03 | 4.2 |
| High in P (0.53%) | 0.96 | 0.89 | 0.07 | 7.3 |
| Low in Ca (0.19%) | 0.19 | 0.19 | 0.00 | 0.0 |
| Low in Ca (0.26%) | 0.26 | 0.28 | 0.02 | 7.7 |
| High in Mo (1072 p.p.m) | 1.24 | 1.15 | 0.09 | 7.3 |
| High in Mo (611 p.p.m) | 0.41 | 0.42 | 0.01 | 2.4 |
| High in Mo (811 p.p.m) | 0.70 | 0.66 | 0.04 | 5.7 |
| High in N (5.64%), P (0.51%) and Fe (251 p.p.m) | 0.83 | 0.86 | 0.03 | 3.6 |
| High in N (5.50%), P. (0.57%) and Fe (258 p.p.m) | 0.95 | 0.97 | 0.02 | 2.1 |
| High in N (5.37%), P (0.47%) and Fe (294 p.p.m) | 0.92 | 0.96 | 0.04 | 4.3 |
| High in N (5.46%), P (0.52%) and Fe (286 p.p.m) | 1.05 | 1.02 | 0.03 | 2.9 |
| High in P (0.51%) and Fe (283 p.p.m) | 0.96 | 0.99 | 0.03 | 3.1 |

Several methods for eliminating the interferences of other elements were tried. A method, in which calcium is precipitated as the oxinate and then dissolved in dilute acid before determination (Beaufils 1954), though a definite improvement, gave rather low results and the error often exceeded the 10% limit. The lower values (than those obtained by a chemical method) were attributed to the presence of magnesium and phosphate ions which were also precipitated with the calcium under the conditions of the oxinate separation.

Ion exchange resins may be employed to remove phosphate ions from solutions whose calcium content is due to be assayed by flame photometric means (Hemingway 1956). However, our experiences showed that even the use of Zeokarb 215 and Amberlite IR120 permitted errors which exceeded 10% and 30%, respectively; generally, these errors were greater in the case of leaves obtained from soil grown plants than for those grown in sand. The use of an anion exchange resin, Deacidite B, gave abnormally low values and was also abandoned.

Success was finally achieved by precipitation of calcium from the wet digest, as the oxalate; this precipitate is dissolved in dilute hydrochloric acid and used for the flame analysis. A similar method has been reported (Williams & Morgan 1953). Prior separation of phosphate is unnecessary and the procedure can be applied to both soil and sand grown leaf material. The values obtained deviate up to 10% from values obtained by a well tried purely chemical method. The flame method is now being used on a routine basis at a wavelength of 554 millimicrons and the detailed procedure finally evolved is as follows.

A 20 ml aliquot of the wet digest solution is transferred into a 100 ml centrifuge tube and the following reagents added: 2 drops of methyl red indicator, 5 ml of 30% (w/v) ammonium chloride solution and 2.5 ml of 10% (v/v) acetic acid. The tube is warmed on a boiling steam bath for about 10 minutes and 5 ml of warm 5% (w/v) ammonium oxalate solution added. Ammonium hydroxide (approximately 5N) is added dropwise until the solution is alkaline. The tube is warmed on a boiling steam bath for at least thirty minutes and subsequently cooled overnight. As much as possible of the supernatant liquid is withdrawn by suction, without disturbing the calcium oxalate precipitate; the suction tube is washed clean with hot water and the volume made up to approximately 30 ml. The tube is centrifuged at 2,000 r.p.m. for five minutes, the supernatant liquid removed and the precipitate dissolved in 2.0 ml of 5N hydrochloric acid. The solution is carefully transferred to a 10 ml graduated flask and made up to the mark with water. The solution is then ready for flame analysis. The Beckman spectrophotometer and flame attachment controls are set as follows:

| | |
|---|---|
| Slit width .. .. .. | 0.25 mm |
| Panel oxygen pressure .. | 10 p.s.i. |
| Panel acetylene pressure .. | 5 p.s.i. |
| Phototube knob .. .. | 'all out' (blue phototube) |
| Load resistor switch at | No 3 (10,000 megohms) |
| Selector switch at .. .. | 0.1 |

The transmittance scale is set to 100%, using the luminosity of a 300 p.p.m. calcium standard; the setting is checked after every three determinations. The working range of the method extends between 0 and 300 p.p.m. of calcium but the best results are

usually obtained in the range between 100 to 200 p.p.m. The method, as outlined above, applies to the wet digest solutions derived from leaf material; for digests prepared from roots, which often contain a large amount of iron, a preliminary removal of iron is necessary to achieve correct values.

Table 2 gives a comparison of values obtained by both chemical and flame photometric methods of analyses on the same wet digests, derived from leaf samples of varied mineral status. The correspondence between methods is good. The chemical method involves a dry ashing of the laminae, followed by solution in hydrochloric acid; the calcium is precipitated as oxalate, separated, and estimated by titration against permanganate. The whole operation from the dry leaf powder stage usually permits the completion of two dozen estimations in about one and a half working days. The flame photometric procedure reduces the time needed to assay the calcium, from the calcium oxalate stage. But the biggest advantage is that it can be carried out directly on the wet digest whereas the chemical method requires an independent dry ashing procedure, for which a minimum 0.5 gm of dry lamina powder is needed.

### ESTIMATION OF PHOSPHORUS

Phosphorus is determined as described by Truog & Meyer (1929); the estimation is based on the measurement of the light absorption of the reduced phosphomolybdate blue complex. One modification which has been introduced is to bring the wet digest to a pH of 4.0 before adding the reagents listed by Truog and Meyer. The method covers a range of 0 to 30 micrograms of phosphorus and requires 0.5 to 1.0 ml aliquot of the wet digest. Almost invariably it has been found that if a known amount of phosphorus is added to leaf material, before wet digestion, the proportion recovered by estimation ranges between 95 to 105% of the amount added.

The preparation of reagents is given by Truog & Meyer (1929). The principal details of the method, as adopted by us, are as follows. One millilitre of the wet digest solution is diluted to approximately 75 ml, with water, in a 100 ml graduated flask. Two drops of beta-dinitrophenol indicator (0.1 gm in 2.5 ml of ethanol plus 97.5 ml of water) are added and brought to neutrality with, approximately, 2N ammonium hydroxide. Two millilitres of the ammonium molybdate and sulphuric acid reagent are added and the solution made up to 100 ml before 0.4 ml of the stannous chloride reagent is added. The flask is well shaken and allowed to stand for precisely three minutes. The light absorption of the blue solution is measured by means of Hilger Biochem absorptiometer, using a red filter (No 70) and 2 cm cells.

Usually one laboratory assistant can complete comfortably, from the wet digest stage, two dozen estimations within one half of a working day.

### ESTIMATION OF MAGNESIUM

A method of magnesium estimation (Hunter 1950), based on the formation of a coloured complex of magnesium hydroxide with thiazol yellow dye, has been found to be fully satisfactory and is used by us without modification. A 3 to 5 ml aliquot of the wet digest is required for the estimation and a working range of 20 to 100 micrograms of magnesium is covered. Known amounts of magnesium added to the leaf material, before acid digestion, can be recovered to within 5% of the amount added.

The reagents and their preparation have been listed by Hunter (1950). Briefly the method of estimation is as follows. A suitable wet digest aliquot is pipetted into a 100 ml capacity conical flask, evaporated to dryness and the residue dissolved

in 15 ml of Morgan's reagent. Oxalic acid is added and the flask stood for one hour before the tartarate reagent, dye reagent and sodium hydroxide are added successively; the flask is shaken between each addition, and the final solution is allowed to stand overnight. The dye, which is not complexed by magnesium, is removed by shaking with a mixture of butan-l-ol and ethanol; the light absorption of this unused dye solution is measured in a Spekker absorptiometer. Blue green filters (No 3) and 4 cm cells are used for this purpose.

One laboratory assistant is able to complete two dozen estimations of magnesium, on the wet digest solutions, within the space of one to one and a half working days.

### ESTIMATION OF MANGANESE

This estimation requires a larger aliquot of the wet digest than for any of the other elements discussed here. The method is based on procedures described by Willard & Greathouse (1917) and Richards (1930) and depends on the oxidation of manganese to permanganate, which is then estimated absorptiometrically. The method has been well tried and recovery values obtained for known amounts of manganese added to leaf material, before digestion, have been consistently good (95% to 105%). A range of 0 to 100 micrograms of manganese is covered by the method. A suitable aliquot (25 to 50 ml) of the wet digest solution is placed in a 100 ml beaker flask, 3 ml of syrupy phosphoric acid (sp. gr. 1.75) added, and the mixture evaporated on a hot plate until most of the fumes of perchloric acid (left over from the wet digestion) are driven off. The flask is cooled, about 25 ml of water added, followed by 0.2 gm of potassium periodate, and the solution boiled on a steam bath. Boiling is continued for one hour during which period the maximum purple colour development occurs. The flask is cooled and its contents diluted to 25 ml in a graduated flask. Manganese standards are prepared simultaneously in identical fashion and their light absorption values measured by means of Hilger Biochem absorptiometer, using a blue green filter (No 52) and 4 cm cells.

The completion of two dozen determinations requires the attention of one laboratory assistant for one working day but during that period he is not fully occupied and can carry out other duties.

### DISCUSSION

The aim of this work was to evolve or modify methods by means of which it is possible to analyse about four dozen samples of rubber leaves for potassium, calcium, phosphorus, magnesium and manganese within the period of four days. This limited objective was achieved; three laboratory assistants are necessary for the routine implementation of this objective. One assistant is responsible for the wet digestion of the leaf samples and the analysis of the digests for phosphorus; another assistant is responsible for the estimation of potassium and calcium; and the third assistant is concerned entirely with the estimation of magnesium and manganese. The improvement in the rate of production of analytical results, which amounted to a 50% increase over the previous rate, was due entirely to the introduction of flame photometric methods of assay for potassium and calcium. The use of the flame photometer not only reduced the time required for each estimation but enabled all the elements to be determined on the basis of one wet digest rather than having a separate dry ashing procedure for calcium.

The methods, as described here, give consistent and reproducible results. The chemical methods satisfy the criterion that a weighed amount of standard when added

to the dry leaf material can be recovered to the extent of ± 5% of the amount added. The adaptation of the flame photometric methods for routine purposes was adjudged on whether or not they gave an analysis value, for a leaf sample, to within 10% of that obtained by the chemical method formerly employed. Many papers are published which describe methods of estimating various elements in plant material; frequently we find that we cannot achieve the precision and accuracy claimed by the authors of such papers under routine conditions. The value of our present paper lies in the fact that methods are described which have been well tested and are used by us under routine circumstances, for the analysis of rubber leaves. For all our purposes we regard values which are within 10% of the true values as being of sufficient accuracy in the analysis of hevea leaf material. While more accurate but slower procedures may be available, it is satisfactory to us that three laboratory assistants can analyse four dozen leaf samples, for five different elements in less than a week.

All the methods have been mainly directed towards the analysis of hevea leaf material. It is not expected that difficulties would be encountered with any other type of leaf material but it is known, from our experience, that for roots, iron must be removed before calcium may be estimated accurately by the flame photometric method.

Besides the estimation of the five elements discussed above, the following elements are also determined by us when required: nitrogen, sulphur, iron, boron, copper, zinc and molybdenum. Routine chemical methods are available for each of these estimations but each procedure demands a separate dry or wet ashing of the leaf material. These procedures require the following weights of lamina powder: nitrogen 0.05 gm, boron and iron 0.5 gm each, zinc 1gm, sulphur, copper and molybdenum 2 gm each. Thus these elements represent a heterogeneous group and require varying sub-samples of the original lamina powder, whereas the five elements discussed herein represent a discrete group and are all determined on the same wet digest solution.

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*May 1957*

REFERENCES

BAUSERMAN, H. M. & CERNEY JR, R. R. (1953) Flame spectrophotometric determination of sodium and potassium. *Analyt. Chem. 25*, 1821.

BEAUFILS, E. R. (1954) Méthodes d'analyse utilisées à l'I.R.C.I. pour le dosage de neuf éléments minéraux dans les latex et les feuillages. *Caoutchouc 12*, 5.

BLACKWELL, A. T., YEAGER, C. L. & KRAUS, M. (1953) Use of flame photometry to determine potassium at 404 millimicrons. *J. Ass off. agric. Chem. Wash. 36*, 898.

BOLLE-JONES, E. W. (1954) Nutrition of *Hevea brasiliensis* : I. Experimental methods. *J. Rubb. Res. Inst. Malaya 14*, 183.

HEMINGWAY, R. G. (1956) The determination of calcium in plant material by flame photometry. *Analyst 81*, 164.

HUNTER, J. G. (1950) An absorptiometric method for the determination of magnesium. *Analyst 75*, 91.

Richards, M. B. (1930) The colorimetric determination of manganese in biological material. *Analyst* 55, 554.

Roy, N. (1956) Flame photometric determination of sodium, potassium, calcium, magnesium, and manganese in glass and raw materials. *Analyt. Chem.* 28, 34.

Toth, S. J., Prince, A. L., Wallace, A. & Mikkelsen, D. S. (1948) Rapid quantitative determination of eight mineral elements in plant tissue by a systematic procedure involving use of a flame photometer. *Soil Sci.* 66, 459.

Truog, E. & Meyer, A. H. (1929) Improvements in the deniges colorimetric method for phosphorus and arsenic. *Industr. Engng Chem. (Anal.)* 1, 136.

Willard, H. H. & Greathouse, L. H. (1917) Colorimetric determination of manganese by oxidation with periodate. *J. Amer. Chem. Soc.* 39, 2366.

Williams, T. R. & Morgan, R. R. T. (1953) The determination of calcium in plant material by the flame photometer. *Chem. & Ind. (Rev.)*, 970.

# *Effects of Mineral Status and Light on Rubber Formation in Hevea*

E. W. BOLLE-JONES and V. R. MALLIKARJUNESWARA

*The concentration of rubber and phosphorus in the stems of very young rubber seedlings increased with increased supply of phosphate. Later, when phosphate status markedly affected the dry weight of each seedling, this relationship disappeared and the rubber concentration decreased with increased seedling dry weight. More rubber per seedling was formed in the light than in the dark. The effect of light on rubber formation was more important than that of mineral status, the influence of which was indirect. The amount of rubber found in each seedling was proportional to the amounts of dry matter, carbohydrates and proteins present.*

THE APPLICATION OF FERTILISERS to *Hevea brasiliensis* in the field may increase the yield of rubber produced per tree (HAINES 1946, OWEN, WESTGARTH & IYER 1957); this increase is believed due to the enhanced growth and vigour of the tree and the consequent greater output of latex. The concentrations and balance of the nutrient elements in leaves and latex may influence the yield of rubber per tree (BEAUFILS 1956) but information, reviewed below, on the effect of nutrient elements on the concentration of rubber in plant tissues is scant.

The concentration of rubber in the stems of soil grown guayule seedlings, *Parthenium argentatum*, was smaller at the higher level of available nitrogen supply than at the lower level (BENEDICT 1950). The concentration of rubber in the leaves of the same plant decreased as the proportion of ammonium ions, supplied in nutrient solution, increased; a high nitrate type of nutrient produced the highest concentration of rubber in the leaves (BONNER 1944). Reduction in the concentrations of rubber in the stems and roots of guayule and kok saghyz plants, due to boron deficiency, have also been noted (MITCHELL, BENEDICT & WHITING 1944, MEYER 1946).

It has been shown that a deficiency of any one of the nutrient elements decreased the percentage concentration of rubber in the stems and petioles of very young (up to four months old) *Hevea brasiliensis* seedlings grown in sand culture (BOLLE-JONES 1954a); but with continued growth, a reversal occurred and the healthy plants contained a smaller percentage concentration of rubber but grew much larger than the deficient plants. In a subsequent experiment (BOLLE-JONES 1954b) the effects of different levels of magnesium, potassium and phosphorus on the concentration of rubber in the stems and petioles of *Hevea brasiliensis* were found to be inconsistent from month to month. Significant concentration increases were obtained but they were never large (not more than 25%); their small magnitude suggested that the concentration of rubber within the plant was not directly affected by the nutrient concentration, but only the total amount produced per plant. The dry weight of the shoot appeared to be inversely related to the concentration of rubber found in the stems and petioles.

In another investigation, concerned with field grown *Hevea* plants, it was observed that the petiolar rubber concentration varied according to the concentration of nitrogen found in the laminae (BOLLE-JONES & RATNASINGAM 1954). Later it was found (BOLLE-JONES 1955), for *Hevea brasiliensis* seedlings grown in sand, that as the nitrogen concentration of the laminae increased the rubber concentration decreased. Increased nitrate level significantly decreased the concentration of rubber found in bulked stems and petioles.

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There was, therefore, evidence which indicated that the mineral nutrient status of the plant might affect the concentration of rubber found in the tissues. These effects were not always explicable and sometimes appeared to be contradictory but they did allow the inference that in the earlier stages of growth, before the dry weight effects due to differential mineral nutrient treatments became marked, increases in the concentration of rubber in the tissues might be obtained with improved mineral status. They also indicated that the total dry weight of the shoot of *Hevea brasiliensis* might be inversely related to the concentration of rubber in the stems and petioles and that different effects might be obtained according to the tissue analysed.

Most of the foregoing observations involved a comparison of foliar composition with the concentrations of rubber in the laminae or stems and petioles. However, it chanced in 1955 that analysis of hevea stem tissue for both minerals and rubber was carried out by the senior author and that an approximately linear relationship between the concentration of phosphorus and rubber was revealed. The first part of this paper describes an experiment designed to confirm and amplify this chance finding. It was found that a rough proportionality existed between the concentrations of phosphorus and rubber in the stems of plants, up to the four months old stage; but this relationship disappeared as the plants grew older and their dry weights varied widely among treatments. The effect of phosphorus was not specific as the same type of relationship appeared to hold in respect of the stem concentrations of other nutrient elements also.

It was suspected, even before these results became available, that as far as the investigation of the effects of nutrient elements on the concentration of rubber in tissues of pot grown plants was concerned valid comparisons could only be made in the absence of any marked plant dry weight changes and that any future investigations must involve very short term experiments. It was decided to adopt the alternative procedure of assaying the total rubber and certain other organic fractions within the plant and to attempt to relate them to nutrient treatment.

The objective of this latter approach was to determine the means by which mineral nutrient treatment affected the total rubber content per plant. While it was reasonably certain that none of the nutrient elements exercised a specific effect on the concentration of rubber in the tissues (so that in the absence of such an element the concentration of rubber would be greatly reduced and that this effect could not be brought about by any other element) it was not established with certainty that the formation of rubber was a mere consequence of growth and could only be influenced by any factor which, in the first instance, affected growth itself and the production of plant dry matter.

It was desirable to establish some indication of how rubber formation could be influenced by mineral nutrient status. With this objective an experiment was initiated in which the effects of mineral nutrient elements and also of light intensity on the production of rubber, carbohydrate and protein fractions were examined. The results, which are reported below, showed that:

a the effect of mineral status on rubber formation was an indirect one;

*b* the effect of light was more pronounced than that of mineral treatments and that more rubber per plant was formed in the light than in the dark;

*c* the rubber content per plant, whether in light or darkness, bore a linear relationship to the dry weight of plant, total carbohydrates per plant and total proteins per plant.

This preliminary study (Bolle-Jones & Mallikarjuneswara 1957) will serve as the basis for later studies on the elucidation of the effect of mineral nutrient elements on the formation of rubber in *Hevea brasiliensis*.

## RELATIONSHIP BETWEEN CONCENTRATIONS OF PHOSPHORUS AND RUBBER IN THE STEM

### *Experimental*

Seedlings of clone Tjirandji 1 were grown in sand contained in five gallon bitumen painted clay pots. The sand (particle size, 0.5 to 1.2 mm) was purified by washing with cold dilute hydrochloric acid (2% w/v) and subsequently restored to neutrality by leaching with nutrient salt solutions, as used for each respective treatment.

Phosphate was applied at five different levels which were 0.6 ($P_1$), 1.2 ($P_2$), 2.4 ($P_3$), 4.8 ($P_4$) and 9.6 ($P_5$) milligram equivalents per litre. In addition to phosphate, and an adequate supply of the micronutrient elements, each nutrient solution contained the following concentrations of the other essential elements (as m.eq./l): $SO_4^{--}$ 6.3 to 9.3, $NO_3^-$ 5, $NH_4^+$ 3, $Mg^{++}$ 2.5, $K^+$ 3, $Ca^{++}$ 4, $Na^+$ 2, $Fe^{+++}$ 0.66. Each of the five phosphate treatments was duplicated and each replicate contained four pots. Eighteen seeds, each weighing more than 5 grams, were sown in each pot on 31 August 1956. Complete plants were successively removed from each pot at 60, 95, 131, 192 and 235 days after sowing. For the earlier samplings a minimum of three plants was removed from each pot until only one remained to be taken at the last sampling.

All the stems removed at the first two samplings were analysed, but for subsequent samplings the green portion only of each stem was used and the brown lower portion discarded. The brown portion was discarded in order to avoid the inclusion of an increasing proportion of non rubber bearing tissue in the material to be analysed and the consequent lower values which would be obtained for the rubber concentration values. The stem material was analysed for rubber (Meeks, Crook, Pardo & Clark 1953), phosphorus, nitrogen, magnesium, potassium and calcium (Bolle-Jones, Mallikarjuneswara & Ratnasingam 1957); the total dry weight per plant per replicate was also recorded.

### *Results*

Plants which received the $P_1$ nutrient showed typical visual symptoms of phosphorus deficiency (Bolle-Jones, 1956) in March; their growth became thin and retarded. Plants grown at the highest phosphate level ($P_5$ treatment) appeared paler and smaller than those of the $P_3$ treatment, which roughly corresponded to the phosphate level required for normal growth; but towards the end of the experiment they appeared to gain in relative vigour. Increased phosphate level caused an increased proportion of the lower leaves to develop a yellow mottling, especially near the tips and edges. In general, the heaviest dry matter production was obtained for plants grown at the $P_3$ level. Plants grown at the higher phosphate level ($P_3$ and $P_4$) usually produced a smaller dry weight during the period covered by this experiment.

The concentration of phosphorus in the stem increased with applied phosphate level. The concentration of rubber in the stems increased with increases in the stem phosphorus concentration up to and including samples taken 131 days after sowing (*Figure 1*). Later samplings showed that the concentration of rubber in the stems of the $P_2$ and $P_3$ plants were smaller than for the $P_1$ plants. Examination of the relevant graphs shows that a decrease in stem rubber concentration was usually, but not always, accompanied by an increase in the dry weight of the plant. A retarded growth, as was apparent in the phosphorus deficient plants, produced an increase in the stem rubber concentration. However, the beneficial effect of phosphate level on rubber concentration during the earlier samplings, cannot be solely attributed to the increased concentration of phosphorus found in the stems as concomitant increases in nitrogen, magnesium, potassium and calcium concentrations were also recorded (Table 1).

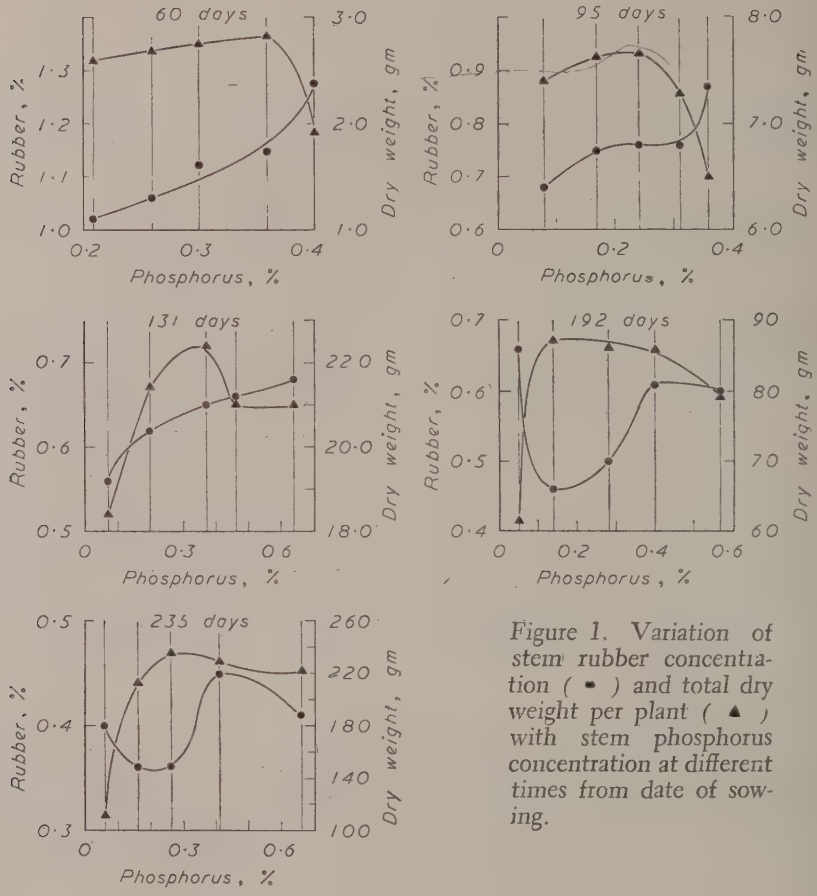


*Figure 1. Variation of stem rubber concentration ( ▪ ) and total dry weight per plant ( ▲ ) with stem phosphorus concentration at different times from date of sowing.*

The general relationship found between stem rubber concentration and plant dry weight is represented in *Figure 2*. An increase in plant dry weight markedly reduced the concentration of rubber found in the green stems. This form of presentation requires qualification as all the values represented were not obtained at any one sampling but they were taken at different sampling times. However, the graph does serve to show that increased dry weight per plant was accompanied by a diminution of the concentration of rubber in the stems.

*Conclusion*

The results fully confirmed the tentative inferences which could be deduced from the literature discussed in the introduction to this paper. They proved that in the earlier stages of growth, during which the mineral nutrient treatments had not promoted any marked differences in terms of dry weight per plant, improved nutrient status increased the concentration of the rubber found in the tissue. This increased rubber

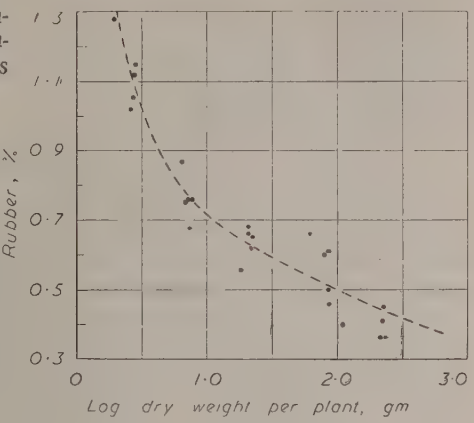


*Figure 2. Illustration of general relationship observed between stem rubber concentration and total dry weight of plant; values derived from seedlings of different ages.*

concentration could not be specifically related to an increased concentration of any one nutrient element. Once large plant dry weight differences became obvious the stem rubber concentration did not increase with increased phosphate level but rather the reverse. Any factor which tended to limit or retard growth, such as deficient or excessive phosphate supply, increased the concentration of rubber found in the stem.

TABLE 1. MEAN CONCENTRATIONS OF NITROGEN, MAGNESIUM, POTASSIUM AND CALCIUM IN GREEN PORTION OF STEMS OF DIFFERENT AGES

| Age | 60 days | | | | 95 days | | | | 131 days | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | N | Mg | K | Ca | N | Mg | K | Ca | N | Mg | K | Ca |
| $P_1$ | 1.38 | 0.14 | 0.82 | 0.37 | 0.99 | 0.16 | 0.70 | 0.48 | 0.91 | 0.20 | 0.92 | 0.62 |
| $P_2$ | 1.34 | 0.14 | 0.88 | 0.42 | 1.12 | 0.17 | 0.88 | 0.51 | 1.24 | 0.27 | 1.14 | 0.67 |
| $P_3$ | 1.29 | 0.14 | 0.90 | 0.40 | 1.12 | 0.19 | 0.92 | 0.54 | 1.20 | 0.28 | 1.35 | 0.65 |
| $P_4$ | 1.32 | 0.14 | 0.87 | 0.48 | 1.18 | 0.20 | 0.96 | 0.56 | 1.18 | 0.28 | 1.34 | 0.66 |
| $P_5$ | 1.49 | 0.16 | 0.93 | 0.51 | 1.20 | 0.19 | 0.91 | 0.58 | 1.32 | 0.31 | 1.56 | 0.73 |

| Values expressed as % of dry matter | Age | 192 days | | | | 235 days | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Treatments | N | Mg | K | Ca | N | Mg | K | Ca |
| | $P_1$ | 0.85 | 0.22 | 0.58 | 0.52 | 0.85 | 0.23 | 0.66 | 0.62 |
| | $P_2$ | 1.08 | 0.34 | 0.94 | 0.60 | 1.16 | 0.32 | 0.90 | 0.66 |
| | $P_3$ | 1.14 | 0.36 | 1.13 | 0.58 | 1.12 | 0.32 | 0.78 | 0.66 |
| | $P_4$ | 1.25 | 0.28 | 1.27 | 0.58 | 0.94 | 0.32 | 1.04 | 0.70 |
| | $P_5$ | 1.13 | 0.42 | 1.24 | 0.58 | 0.96 | 0.38 | 1.20 | 0.70 |

*Experimental*

Tjirandji 1 rubber seedlings were grown in acid washed sand; four different nutrient treatments were applied:

1 Complete nutrient (Compt.);
2 Nutrient solution free of all the micronutrient elements (– mn);
3 Phosphorus free nutrient (– P);
4 Water only ($H_2O$).

All treatments were duplicated and each replicate contained three pots in each of which twenty seeds were sown. One such set of replicated treatments was placed in the light and another in the dark. The sand (particle size 0.5 to 1.2 mm) was purified by boiling with a mixture of hydrochloric acid (4% w/v) and oxalic acid (1% w/v) for seven hours. After leaching free of residual acid, the sand was contained in five gallon bitumen painted clay pots. The complete nutrient solution, on which treatments 2 and 3 were also based, possessed the following composition (in milligram equivalents per litre): $NO_3^-$ 5, $NH_4^+$ 3, $SO_4^{--}$ 7.5, $PO_4^{---}$ 3, $K^+$ 3, $Mg^{++}$ 2.5, $Ca^{++}$ 4, $Na^+$ 1, $Fe^{+++}$ 0.66, $Mn^{++}$ 0.02, $BO_3^{---}$ 0.067, $Cu^{++}$ 0.002, $Zn^{++}$ 0.002, Mo(hexavalent) 0.001, $Al^{+++}$ 0.001, $Ni^{++}$ 0.0002, $Co^{++}$ 0.0002, $Ga^{+++}$ 0.0002.

The solutions containing the macronutrient elements were purified of micronutrient elements by complexing them with alpha-nitroso beta-naphthol and adsorption of the complexes on carbon followed by extraction with dithizone in carbon tetrachloride. Iron, as acidified ferric chloride, was repeatedly extracted with ether, prior to conversion to ferric citrate. Demineralised water was used to supply the plants grown in the – mn and $H_2O$ treatments and tapwater for the remainder. Seedlings grown in the dark received a daily eight hour illumination of two footcandle intensity from an artificial source, whereas those grown in the light, actually light shade, received about 800 foot candles. The seeds, each heavier than 5 grams, were sown in each pot on 31 August 1956. Seedlings, were removed from each pot at 31, 47, and 66 days after sowing. The seedlings, each of which was removed whole, were killed, dried at 55 to 60°C, milled and then analysed for rubber, hydrolysable carbohydrate, crude fibre, and protein (including free amino acids).

At the first sampling the seed residues were separated from the seedlings and both were analysed separately. The seed residues of the plants not sampled were also excised at the first sampling (and discarded) to facilitate the convenient interpretation of the results. Samples of the original seed supply as well as the seed residues were analysed for their fat content as well for the other fractions mentioned. All the results are expressed as contents of the constituent or fraction per seed, seed residue or seedling.

*Rubber* — This was determined by a method based on that of Meeks, Crook, Pardo & Clark (1953).

*Hydrolysable carbohydrates* — These, including the 'hemicelluloses', were estimated as follows. One gram of dried plant material was hydrolysed with 50 ml of 1 N hydrochloric acid at 70 to 75°C for a period of 3½ hours. An aliquot of the filtrate was deproteinised and cleared with zinc sulphate and barium hydroxide solutions before its reducing sugar content was estimated in the usual manner with a copper sulphate and tartarate reagent. A set of invert sugar standards was always determined alongside every set of unknowns. The results are expressed as milligrams of invert sugar per seedling, seed or seed residue.

*Crude fibre* — The hydrochloric acid insoluble residue obtained during the estimation of the hydrolysable carbohydrates was boiled with 50 ml of 1.25% (w/v) sodium hydroxide for thirty minutes. The crude fibre value was obtained as the difference between the weights of the insoluble fraction before and after ignition; this value was divided by 0.9 so that the results could be expressed as milligrams of invert sugar.

*Total carbohydrate* — This value was obtained by summing the values for the hydrolysable carbohydrate and crude fibre fractions.

*Protein content* — An estimate which included any free alpha-amino acid originally present in the material was obtained after the hydrolysis of the plant material by estimation of the alpha-amino nitrogen with a ninhydrin reagent. Half a gram of the plant material sample was hydrolysed with 10 ml of 8 N hydrochloric acid at 80 to 85°C for eight hours. The solution was cooled and made up to 100 ml with water and then filtered. A 1 ml aliquot was taken, ninhydrin and other reagents were added, and the resultant colour was measured absorptiometrically. A description and preparation of the respective reagents and the mode of colour development were described by Wiggins & Williams (1955). A series of glycine standards was run with each batch of determinations. Glycine was chosen for the standard as it was found in our preliminary trials that six out of the nine acids tested gave ninhydrin complexes whose absorption was close to that of glycine. To obtain a protein value for the plant material sample the concentration of alpha-amino nitrogen was multiplied by 6.25 and the results were expressed as milligrams of proteins (including free amino acids) per seedling, seed or seed residue.

*Fatty oil* — An estimate of the content of seeds and seed residue was obtained by soxhlet extraction of the powdered sample with dry ether for eight hours. The ether solution was removed, the ether evaporated and the residual fatty oil weighed after heating to a constant weight at 100°C. Carbohydrates were later estimated on the ether extracted seed and seed residue material.

*Results*

The plants grown in the dark became drawn and etiolated while those deprived of all nutrients, but grown in the light, also appeared pale and nitrogen deficient. Although no symptoms which could be attributed to a mineral deficiency were noted, the Compt and – P plants were, at the six weeks stage, 10 to 20% taller than those of the other treatments; that is, a lack of the micronutrient elements produced shorter plants.

Dry weight and analytical data for the third sampling were incomplete, owing to the lack of plant material for the $H_2O$ (light), – mn (dark) and $H_2O$ (dark) treatments. The dry weights of the dark grown plants varied little over the duration of the experiment but those of the light grown plants increased with time, according to mineral nutrient treatment. The effect of light on plant dry weight was greater than that of the nutrient treatment and was significant even at the earliest sampling whereas the nutrient treatments did not show significance until the second sampling (Table 2). The dry weights of the Compt. and – P plants were greater than those of plants grown in the – mn and $H_2O$ treatments. Similar trends were observed for the rubber, hydrolysable carbohydrate, crude fibre, total carbohydrate, and protein contents, per plant. More of each constituent was found in plants of the same mineral nutrient treatment grown in the light than in the dark.

The rubber content became reduced in plants grown in the – mn and $H_2O$ treatments (Table 2); this reduction was significant in the – mn (light) plants at the third sampling. The hydrolysable carbohydrate, but not the crude fibre content, was affected by nutrient treatment at the first sampling (Table 3). Plants which did not receive phosphate (– P and $H_2O$ treatments) contained more hydrolysable carbohydrates than

those which did. A similar effect was noted for the third sampling. Much less crude fibre was found in the plants of the – mn and $H_2O$, than in other treatments at the second and later samplings. The total carbohydrate content was, for the second sampling, least in plants grown in the – mn treatment; values for plants given the $H_2O$ treatment were also much reduced. Plant protein content was not significantly influenced by mineral treatment at the first sampling (Table 2). Later for the second

TABLE 2. DRY WEIGHT, RUBBER AND PROTEIN CONTENTS PER PLANT

*Dry weight, grams*

| Sampling time | Light or dark | Nutrient treatment | | | | Min. 5% sig. diff. |
|---|---|---|---|---|---|---|
| | | Compt. | – P | – mn | $H_2O$ | |
| 31 days | L | 1.14 | 1.25 (±0.050) | 1.25 | 1.21 | 0.169 |
| | D | 0.82 | 0.98 | 0.76 | 0.81 | |
| 47 days | L | 1.63 | 1.50 (±0.065) | 1.14 | 1.26 | 0.218 |
| | D | 0.90 | 0.90 | 0.58 | 0.70 | |
| 66 days | L | 2.80 | 3.30 (±0.147) | 2.17 | — | 0.893 |
| | L | 2.80 (±0.055) | 3.30 | — | — | 0.248 |
| | D | 1.04 | 1.04 | — | — | |

*Rubber, milligrams*

| Sampling time | Light or dark | Nutrient treatment | | | | Min. 5% sig. diff. |
|---|---|---|---|---|---|---|
| | | Compt. | – P | – mn | $H_2O$ | |
| 31 days | L | 10.3 | 10.3 (±0.35) | 11.0 | 10.5 | 1.16 |
| | D | 7.5 | 8.0 | 7.7 | 7.6 | |
| 47 days | L | 13.4 | 13.1 (±1.04) | 10.9 | 11.4 | 3.48 |
| | D | 9.1 | 8.9 | 6.7 | 7.5 | |
| 66 days | L | 22.4 | 24.2 (±0.28) | 19.5 | — | 1.72 |
| | L | 22.4 (±1.17) | 24.2 | — | — | 5.27 |
| | D | 11.8 | 10.7 | — | — | |

*Protein, milligrams*

| Sampling time | Light or dark | Nutrient treatment | | | | Min. 5% sig. diff. |
|---|---|---|---|---|---|---|
| | | Compt. | – P | – mn | $H_2O$ | |
| 31 days | L | 81 | 84 (±3.7) | 87 | 77 | 12.3 |
| | D | 57 | 64 | 50 | 53 | |
| 47 days | L | 112 | 110 (±5.5) | 85 | 73 | 18.4 |
| | D | 75 | 74 | 46 | 53 | |
| 66 days | L | 201 | 193 (±16.9) | 149 | — | 103.0 |
| | L | 201 (±3.1) | 193 | — | — | 13.8 |
| | D | 84 | 83 | — | — | |

sampling, it was found that the absence of the micronutrient elements from the nutrient significantly decreased the plant protein content.

For the first and second samplings, there was no evidence of any interaction between light and mineral nutrient treatments. For the third, and incomplete, sampling there

Table 3. Hydrolysable Carbohydrate, Crude Fibre and Total Carbohydrate Contents per Plant

*Hydrolysable carbohydrate, miligrams*

| Sampling time | Light or dark | Nutrient treatment | | | | Min. 5% sig. diff. |
|---|---|---|---|---|---|---|
| | | Compt. | – P | – mn | $H_2O$ | |
| 31 days | L | 214 | 259 (±9.2) | 237 | 300 | 30 |
| | D | 148 | 201 | 150 | 197 | |
| 47 days | L | 335 | 289 (±17.4) | 203 | 304 | 58 |
| | D | 137 | 126 | 88 | 111 | |
| 66 days | L | 484 | 661 (±8.2) | 419 | — | 50 |
| | L | 484 (±10.3) | 661 | — | — | 46 |
| | D | 108 | 127 | — | — | |

*Crude fibre, milligrams*

| Sampling time | Light or dark | Nutrient treatment | | | | Min. 5% sig. diff. |
|---|---|---|---|---|---|---|
| | | Compt. | – P | – mn | $H_2O$ | |
| 31 days | L | 275 | 289 (±12.4) | 309 | 277 | 41 |
| | D | 184 | 190 | 157 | 169 | |
| 47 days | L | 429 | 403 (±20.0) | 325 | 330 | 66 |
| | D | 248 | 246 | 160 | 180 | |
| 66 days | L | 837 | 880 (±36.3) | 534 | — | 221 |
| | L | 837 (±25.0) | 880 | — | — | 112 |
| | D | 322 | 314 | — | — | |

*Total carbohydrate, milligrams*

| Sampling time | Light or dark | Nutrient treatment | | | | Min. 5% sig. diff. |
|---|---|---|---|---|---|---|
| | | Compt. | – P | – mn | $H_2O$ | |
| 31 days | L | 490 | 548 (±20.0) | 546 | 577 | 67 |
| | D | 333 | 391 | 307 | 366 | |
| 47 days | L | 764 | 693 (±31.7) | 528 | 633 | 106 |
| | D | 386 | 372 | 248 | 281 | |
| 66 days | L | 1322 | 1541 (±44.5) | 954 | — | 271 |
| | L | 1322 (±33.5) | 1541 | — | — | 151 |
| | D | 430 | 441 | — | — | |

was some evidence that a beneficial effect due to the – P treatment on hydrolysable carbohydrate content and plant dry weight was obtained in the light but not in the dark.

From the standpoint of interest and record, rather than for the direct purpose of this paper, the significant effects of light and mineral treatments on the dry matter, rubber and other fractions within the seed are shown in Table 4. Neither light nor nutrient treatments influenced the amount of rubber and total carbohydrates removed from the seed by the seedling. There occurred a greater loss of dry matter per seed, and a greater translocation of protein and fatty oils from the seed to the seedling in the light than in the dark. For each of these variables the main effects of nutrient application were observed in the dark treatments. In general, dry weight losses and removal of protein and fatty oils from the seed were much lower in the – mn and $H_2O$, than other, treatments.

*Correlationships between different components and rubber*

When all the available values, regardless of whether they were obtained from light or dark grown plants, were considered, correlationship coefficients significant at the 0.1% level were obtained between rubber and each of the following: dry weight of

Table 4. Amounts of Dry Matter, Protein and Fatty Oil Lost or Translocated from Seed to Seedling

*Seed residues excised 31 days after sowing*

| Fraction | Light or dark | Nutrient treatment | | | | Min. 5% sig. diff. |
|---|---|---|---|---|---|---|
| | | Compt. | – P | – mn | $H_2O$ | |
| Dry matter (gm) | L | 1.19 | 1.26 | 1.24 | 1.12 | 0.114 |
| | D | 1.04 | 1.05 (±0.034) | 0.86 | 0.82 | |
| Protein (mg) | L | 82 | 95 | 87 | 77 | 10.9 |
| | D | 75 | 71 (±3.2) | 59 | 53 | |
| Fatty oil (gm) | L | 0.57 | 0.61 | 0.56 | 0.55 | 0.087 |
| | D | 0.45 | 0.48 (±0.026) | 0.27 | 0.28 | |

Table 5. Correlation Coefficients between Rubber Content and Dry Weight of Plant, Hydrolysable Carbohydrate, Crude Fibre, Total Carbohydrate and Protein Contents per Plant

*r = correlation coefficient* *y = rubber (mg)*

| Rubber (as y) correlated with: | r values | | Light and dark | |
|---|---|---|---|---|
| | Light | Dark | r values | Regression equation |
| x, plant dry weight (gm) | 0.946 *** | 0.847 *** | 0.960 *** | $y = 2.46 + 7.12x$ |
| x, hydrolysable carbohydrate (mg) | 0.917 *** | –0.184 NS | 0.900 *** | $y = 3.85 + 0.032x$ |
| x, crude fibre (mg) | 0.932 *** | 0.956 *** | 0.956 *** | $y = 3.45 + 0.025x$ |
| x, total carbohydrate (mg) | 0.943 *** | 0.849 *** | 0.956 *** | $y = 3.21 + 0.015x$ |
| x, protein (mg) | 0.845 *** | 0.926 *** | 0.901 *** | $y = 2.09 + 0.108x$ |

*** significant at the 0.1% level NS not significant

plant, hydrolysable carbohydrate, crude fibre, total carbohydrate and protein contents (Table 5). If, however, the values were segregated into light and dark treatments all but the hydrolysable carbohydrates maintained this high positive correlation. The rubber and hydrolysable carbohydrate values obtained for the dark grown plants yielded a negative correlationship which was not significant, but corresponding values for the light grown plants allowed of a significant positive correlationship to be obtained.

## GENERAL DISCUSSION

The results showed that under certain conditions mineral nutrient status could influence the concentration of rubber found in the tissues but this response was in all probability an indirect one and mediated through an effect on factors which influenced growth and rubber production. Even where increased stem rubber concentration could be related to improved phosphate supply (*Figure 1*) the effect of this mineral nutrient could hardly be considered specific as its application caused an increase in the stem concentrations of other nutrient elements besides phosphorus (Table 1). Any factor which limited growth, such as deficient or excessive phosphate supply, increased the concentration of rubber found in the stems. Part of the reason why an increased dry weight per plant was accompanied by diminished stem rubber production may have been an increased proportion of non rubber bearing tissues. However, attempts were made to minimise this factor by the selection of the green portion of the stems for the purpose of analysis and the avoidance of the cork thickened brown stem portion. It was also known that the inverse relationship between dry weight and rubber concentration applied not only to stems but also to laminae; thus when the nitrogen concentration of the laminae and dry weight of the plant were found to increase, the rubber concentration of the laminae decreased (Bolle-Jones 1955). It seemed that the proportion of rubber to plant dry matter might decline if growth was vigorous and that a constant bulk proportionality did not necessarily exist. This statement does not, however, preclude the existence of a rigid proportionality in the ratio of rubber to dry matter formed during the initial stages of growth of any one tissue. It may indicate that under conditions of restricted growth, due for example to a nutrient deficiency, the metabolism of the dry matter (perhaps carbohydrate) may be greater and this, not being replenished, makes the concentration of rubber in the tissue appear higher.

The second experiment described clearly revealed the paramount influence of light not only on the dry weight of the plant but on all other fractions examined (Table 2 and 3). The beneficial effects of light on rubber, crude fibre and protein contents were highly significant at the first sampling and the coresponding effects of mineral nutrient treatments were insignificant. These results might suggest that the formation or carbohydrates preceded the formation of rubber or that light was necessary for rubber to be formed. Our present findings indicated tentatively that light, per se, was not required for rubber formation as the rubber content per plant continued to show a slight increase under the 'dark' conditions of our experiment. However this point can be fully resolved by estimating the rubber contents of plants which are kept in the dark and fed with sugars; there is already some evidence to show that the rubber content would increase as a result of such treatment (J. S. Lowe, unpublished data).

If rubber is assumed to be formed directly from carbohydrates the correlationship coefficients (Table 5) demonstrate that it is more likely to be associated with the crude fibre than with the hydrolysable carbohydrate fraction. Other data (Table 3) indicate that although the mineral nutrient treatments significantly affected the hydrolysable carbohydrate fraction at the first sampling they did not cause a parallel variation in rubber content. On a priori grounds, if rubber was formed from carbohydrates it

would be expected that the relationship would be closest to the hydrolysable carbohydrate rather than to the crude fibre fraction. If rubber is to be regarded as a plant food reserve, as has been suggested (SPENCE & MCCALLUM 1935, TEAS 1956), it might be expected not only to show a close relationship to carbohydrate content but also to diminish under conditions of carbohydrate depletion. No such marked diminution was observed by DE HAAN & VAN AGGELEN-BOT (1949) or under the mild starvation conditions of the experiment described here.

We favour the theory that rubber is formed as a by-product of growth, perhaps as a result of anaerobic respiration (RITTER 1954); this allows us to reconcile the close relationships between rubber and protein and rubber and plant dry weight as well as the relationship of rubber to carbohydrate content (Table 5). As a waste by-product, it would be expected that rubber would be formed irreversibly from its precursor and therefore, under conditions of starvation, would not be metabolised within the plant. To determine whether rubber is derived from protein like. or carbohydrate like, fractions may involve future experiments in which the effects of nitrogen deficient nutrient solutions (with and without sucrose) on plant rubber content are studied.

The practical conclusion to be drawn from these present observations is that the formation of rubber in *Hevea brasiliensis* is intimately connected with growth. Any nutritional deficiency which impedes the optimal formation of carbohydrates and proteins within the plant will diminish the amount of rubber formed per plant. Of great interest, was the marked effect of micro-nutrient element supply on the plant's wellbeing. The absence of the essential traces of these elements from the nutrient solution decreased the dry weight of the plant and its carbohydrate and protein contents. As these are closely related to the amounts of rubber formed in the plant, it seems that the fulfilment of the requirement of *Hevea brasiliensis* for micronutrient elements may be an important factor in the realisation of optimal rubber yields under field conditions and they are worthy of further attention. It was also noted that seedlings grown in the dark and supplied with micronutrient elements were able to withdraw significantly higher amounts of proteins and fatty oils from the seed than those which were not so supplied.

## SUMMARY

Seedlings of *Hevea brasiliensis* were grown in sand and supplied with nutrient solutions in experiments to determine the effect of light and mineral nutrient treatments on the formation of rubber in young plants. During the first four months of growth the concentration of rubber in the green portion of the stems increased with improved phosphate supply. This increased concentration was not specifically related to an increased concentration of phosphorus in the tissues as concentrations of other elements also increased.

Once large plant dry weight differences between treatments occurred, the direct relationship between the concentration of rubber and phosphorus in the stem disappeared and any factor which retarded growth increased the concentration of rubber in the stem.

The rubber content of the plant was closely correlated with its dry weight, carbohydrate and protein contents. As light intensity exerted a greater effect on these values than did nutrient status, variation in the latter was of lesser importance than light as a factor in the formation of rubber.

The effect of mineral nutrient status on rubber formation was an indirect one and was concerned with the role of the nutrient elements in the formation of either carbo-

hydrates, proteins or of plant dry matter. Of great significance was the apparent importance of traces of micronutrient elements in this formative role.

The interpretation of the results was facilitated if rubber was regarded as a by-product of growth rather than as a food reserve.

*We thank Mr Wong See Kiong for his care and maintenance of the experimental plants, Mr Wong Siew Wing for his determination of the various organic fractions, and Mr Chin Pong Tow for his statistical computations.*

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*Accepted June*

REFERENCES

BEAUFILS, E. R. (1956) Recherche d'une exploitation rationnelle de l'Hévéa d'après un diagnostic physiologique réposant sur l'analyse minérale de diverses parties de la plante. *Seventh Int. Congr. Soil Sci. Paris*, 1956.

BENEDICT, H. M. (1950) Factors affecting the accumulation of rubber in seedling guayule plants. *Bot. Gaz. 112*, 86.

BOLLE-JONES, E. W. (1954a) Nutrition of *Hevea brasiliensis*. II. Effect of nutrient deficiencies on growth, chlorophyll, rubber and mineral contents of Tjirandji 1 seedlings. *J. Rubb. Res. Inst. Malaya 14*, 209.

BOLLE-JONES, E. W. (1954b) Nutrition of *Hevea brasiliensis*. III. The interrelationships of magnesium, potassium and phosphorus. *J. Rubb. Res. Inst. Malaya 14*, 231.

BOLLE-JONES, E. W. (1955) Comparative effects of ammonium and nitrate ions on the growth and composition of *Hevea brasiliensis*. *Physiol. Plant. 8*, 606.

BOLLE-JONES, E. W. (1956) Visual symptoms of mineral deficiencies of *Hevea brasiliensis*. *J. Rubb. Res. Inst. Malaya 14*, 493.

BOLLE-JONES, E. W. & MALLIKARJUNESWARA, V. R. (1957) Rubber formation in *Hevea brasiliensis* as affected by light and mineral status. *Nature, Lond. 179*, 1199.

BOLLE-JONES, E. W. MALLIKARJUNESWARA, V. R. & RATNASINGAM K. (1957) Flame photometric determination of potassium and calcium and the chemical estimation of phosphorus, magnesium and manganese in leaves of hevea. *J. Rubb. Res. Inst. Malaya 15*, 86.

BOLLE-JONES E. W. & RATNASINGAM, K. (1954) Nutrition of *Hevea brasiliensis*. IV. Interclonal and seasonal variation in composition of leaves. *J. Rubb. Res. Inst. Malaya 14*, 257.

BONNER, J. (1944) Effect of varying nutritional treatments on growth and rubber accumulation in guayule. *Bot. Gaz. 105*, 352

DE HAAN, I. & VAN AGGELEN-BOT, G. M. (1949) De vorming van rubber bij *Hevea brasiliensis*. *Arch. Rubbercult. 26*, 121.

HAINES, W. B. (1946) Manuring Hevea. IV. Conspectus of experimental improvements achieved in mature stands at the end of ten years with a special note on seed production. *Emp. J. exp. Agric. 14*, 182.

MEEKS, J. W., CROOK, R. V., PARDO, C. E. & CLARK, F. E. (1953) Determining rubber hydrocarbon in rubber-bearing plants. *Analyt. Chem. 25*, 1535.

MEYER, B. S. (1946) Effects of various concentrations of boron on the development of *Taraxacum Kok-Saghyz* in sand culture. *Amer. J. Bot. 33,* 204.

MITCHELL, J. W., BENEDICT, H. M. & WHITING, A. G. (1944) Growth, rubber storage and seed production by guayule as affected by boron supply. *Bot. Gaz. 106,* 148.

OWEN, G., WESTGARTH, D. R. & IYER, G. C. (1957) Manuring Hevea: Effects of fertilisers on growth and yield of mature rubber trees. *J. Rubb. Res. Inst. Malaya 15,* 29.

RITTER, F. J. (1954) Biosynthesis of rubber and other 'isoprenoid' compounds. *India Rubb. J. 126,* 7.

SPENCE, D. & MCCALLUM, W. J. (1935) The function of the rubber hydrocarbon in the living plant. *I.R.I. Trans. 11,* 119.

TEAS, H. J. (1956) *Physiology of rubber formation in hevea.* U.S. Dept. of Agriculture, Maryland.

WIGGINS, L. F. & WILLIAMS, J. H. (1955) Amino-acid content of West Indies sugar cane. *J. Agric. Fd. Chem. 3,* 341.

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